SPACE SYSTEMS

VOLUME III

APPENDIX J

STANDING OPERATING PROCEDURES

FOR

PRIMATE ISOLATION STUDY

PREPARED FOR

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MANNED SPACECRAFT CENTER
Houston, Texas 77058

NASA CONTRACT NAS 9-9000

GENERAL ELECTRIC COMPANY
SPACE SYSTEMS ORGANIZATION
BIOSCIENCES OPERATION
VALLEY FORGE SPACE CENTER

GENERAL ELECTRIC

VOLUME III OF IV

Research on Long Term Biological Isolation of Primates and Mice

APPENDIX J

STANDING OPERATING PROCEDURES FOR PRIMATE ISOLATION STUDY

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FOREWARD

All laboratory operations are controlled by existing laboratory Standing Operating Procedures (SOP's) which are to be consulted for all operations. In cases where existing directives do not cover the intended operation, new SOP's must be written.

Any change in Standing Operating Procedures will be made only with the approval of the Project Manager. Approved changes will be noted in the laboratory experimental records and the revised procedures incorporated into this book.

PRIMATE ISOLATION STUDY

STANDING OPERATING PROCEDURES - CONTENTS

SOP NO.	TITLE
8.2	Water
8.2.1	Containers
8.2.2	Filling
8.2.3	Sterility Assurance
8.2.4	Emergency Reserves
8.3	Vitamins
8.3.1	Type
8.3.2	Preparation and Sterilization
8.3.3	Container Filling and Dispensing
8.3.4	Sterility Assurance
8.4	Glassware and Material
8.4.1	Preparation - General
8.4.2	Pre-Packaged Items Received From Vendors Are Accepted as Sterile
8.4.3	Saline Preparation 9.0 ml and 99.0 ml (see also SOP No.3.2.4, 3.3)
8.4.4	Heparin Sodium Preparation
8.4.5	Hockey Stick Spreaders and Stainless Steel Spatula
8.4.6	Screw Cap Blood Collecting Tubes Preparation
8.4.7	70% Isopropanol Preparation (see also SOP No. 8.4.1)
8.5	Record Keeping (see SOP No. 10.1, 10.4, 10.5)
9.	ISOLATORS - PREPARATION AND MAINTENANCE
9.1	General
9.2	Initial Set-Up of Isolators
9.2.1	Preparation of Filters
9.2.2	Installation of Isolator Covers
9.2.3	Leak Testing and Repair of Leaks and Tears

SOP NO.	TITLE
9.2.4	Sterilization of Isolators with Peracetic Acid (see also SOP No. 9.3.5)
9.2.5	Qualification of Isolator Sterility (see also SOP No. 7.1)
9.3	Routine Operating Procedures
9.3.1	Daily Cleaning Procedures (see also SOP No. 7.2.5)
9.3.2	Leak Testing
9.3.3	Repair of Leaks (see also SOP No. 9.2.3)
9.3.4	Isolator Breakdown or Opening Procedures (see also SOP No. 9.2.2)
9.3.5	Preparation and Use of Peracetic Acid (see also SOP No. 9.2.4)
9.3.6	Check List of Isolator Supplies for Bacterial and Serological Samples (see also SOP No. 8.3, 8.4, 9.3.5)
9.4	Material Transfer - In/Out
9.4.1	Food/Water/Vitamins/Isolator Supplies (see also SOP No. 8.1, 9.2.4)
9.4.2	Bacteriological/Serological Assays (see also SOP No. 7.2.5, 9.4.1)
9.4.3	Cleaning Materials (see also SOP No. 9.4.1, 9.4.2)
9.5	Record Keeping (see also SOP No. 10.1, 10.2)
10.	NOTEBOOKS/RECORD KEEPING
10.1	General Considerations
10.2	Animal and Isolator Records (see also SOP No. 9.3.3)
10.3	Bacteriological Data (see also SOP No. 3.2.6)
10.4	Serology/Hematology
10.5	Food/Water/Vitamin Sterility Control
11.	SAFETY PROCEDURES
11.1	General
11.2	Disposal of Viable Cultures and Contaminated Materials (see also SOP No. 8.4)
11.3	Malfunction of Equipment
11.4	Toxic Chemicals - Handling and Personnel Safety (see also SOP No. 9.3.5)
11.5	Miscellaneous

SOP NO.	TITLE
12.	LABORATORY MAINTENANCE/SERVICES
12.1	General
12.2	Dishwashing (see also SOP No. 8.4)
12.3	Equipment Maintenance
12.4	Housekeeping - Daily/Weekly

1. INTRODUCTION

1.1 GENERAL

SOP NO. 1.1
DATE: 4/16/69
REVISION: 12/22/69

This section contains the Standing Operating rocedures (SOP's) of the Gnotobiology Laboratories to be used on the Primate Program, Part A, Contract NAS 9-9000. The checklists and procedural guidelines were written to direct the performance of routine procedures. There is also a brief reference to procedures to be used as guidelines for further experimentation.

These guidelines are by no means complete and will be improved or altered as the situation demands. They are intended to be used as a workable portion of the experimental program and can be up-dated or changed. These procedures are not to be considered "Standard Operational Procedures.

1. INTRODUCTION

1.2 EXPERIMENTAL PROTOCOL FOR STUDY

SOP NO. 1.2
DATE: 4/16/69
REVISION: 12/22/69

INITIAL BASELINE ASSAY - DETERMINATION OF SPECIES

Determination of Aerobic Bacterial Species

1. Monkeys Sampled - All

2. Sites

   - o Feces
   - o Gingiva
   - o Buccal Area
   - o Ocular Area
   - o Groin Area

3. Assay Media - Total Count/Media

   - o Blood Agar
   - o Phenylethylene Agar
   - o MacConkey's Agar
   - o Mannitol Salts Agar
   - o Serum Tellurite Agar
   - o Mycosel Agar
   - o Rogosa's Agar
   - o PPLO Agar
   - o Chocolate Agar

4. Isolation/Pure Colony - Blood Agar Slants

   - o Colonial Morphology
   - o Color
   - o Texture and Size

5. Microscopic Examination for Gram Characteristics
   - o Gram + cocci
   - o Gram - cocci
   - o Gram + bacilli
   - o Gram - bacilli
   - o Other (Fungi, spirochaetes)
6. Secondary Testing - Appropriate Biochemical Test Media for species determination of each isolate as suggested from initial Gram stain characteristics and colonial morphology.

Determination of Anaerobic Bacterial Species

1. Monkeys Sampled - All
2. Sites
   - o Feces
   - o Gingiva
   - o Buccal Area
   - o Ocular Area
   - o Groin Area
3. Assay Media - Total Count/Media

   Blood agar with antibiotic sensi-discs on plates to inhibit growth of strict aerobic bacteria.
4. Isolation/Pure Culture - Blood Agar Slants
5. Microscopic Examination - for Gram stain characteristics
6. Secondary Testing - Appropriate Biochemical Test Medium for species determination of each isolate.

Selection of "Marker" Organisms

On basis of species determination from initial assay and selection of isolator and control monkeys.

Serological Testing

1. Monkeys Sampled
2. Determinations Made
   - o RBC Total Count
   - o WBC Total Count
   - o Differential
   - o Hematocrit
   - o Hemoglobin

WEEKLY ASSAY FOR QUANTITATIVE MONITORING OF MARKER ORGANISMS

Bacteriological Determinations - Aerobic and Anaerobic

1. Monkeys Sampled
   - o Isolator Animals - 4
   - o Control Animals - 2
2. Sites - Same as Initial Assay
3. Assay Media - Same
4. Data Recorded - Total count of each marker organism on appropriate selective agar medium/incubation conditions; continued use of selective assay media (see 3 under Bacteriological Determination - Aerobic, Initial Baseline Assay Determination of Species) to provide for detection of possible numerical increases of non-"marker" bacterial flora, or emergence of hithertofore undetected bacterial strains.

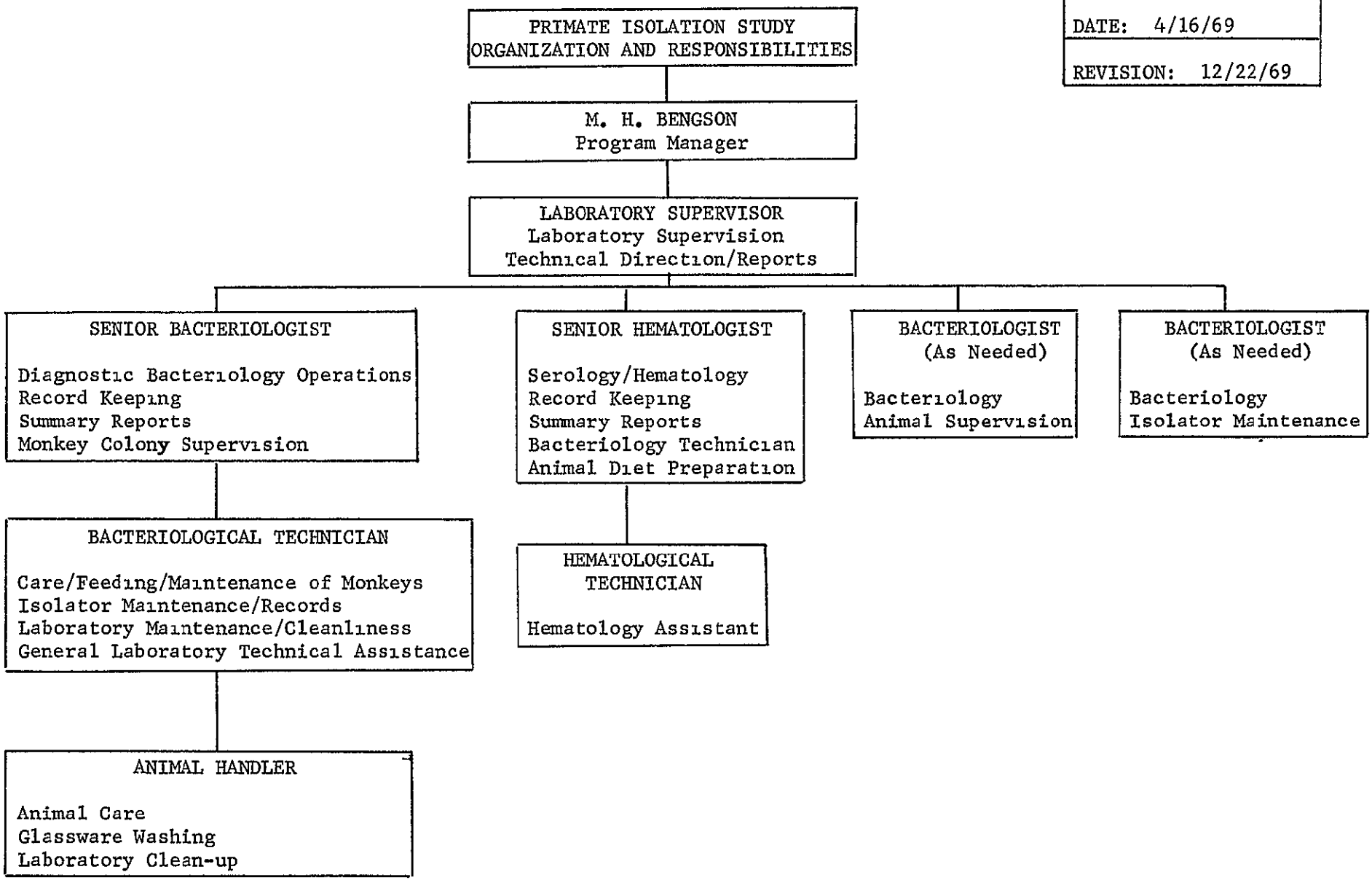
Serological Determinations

1. Monkeys Sampled
   - o Isolator Animals - 4
   - o Control Animals - 2
2. Determination - Same as Initial Assay

ORGANIZATION &
RESPONSIBILITIES

2. ORGANIZATION AND RESPONSIBILITIES

SOP NO. 2
DATE: 4/16/69
REVISION: 12/22/69



3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

SOP NO. 3.1
DATE: 4/16/69
REVISION: 12/22/69

3.1 GENERAL

The procedures contained in this section have been standardized so as to provide a uniform set of operating guidelines which will allow comparison of data over the extent of the sampling period. The standing operating procedures outlined in this section have incorporated the state-of-the-art at the time of their generation and where indicated have been revised accordingly.

They are intended to allow for identification and quantification of the normal indigenous aerobic and anaerobic microflora of the test animals involved. This edition is specific for this program, primate confinement, and should not be indiscriminately applied to other laboratory programs without approval of responsible manager.

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURE

3.2 DETECTION PROCEDURES - AEROBIC/ANAEROBIC

3.2.1 ANIMAL SAMPLING

SOP NO. 3.2.1
DATE: 4/16/69
REVISION:

Body Areas

Each monkey will be sampled at five sites: feces, gingiva, throat, eye, and groin. Polyester-tipped swabs* are rendered wet with 0.85% sterile saline (10 ml/tube) and will be used to sample remaining areas according to the following procedure:

Before withdrawing the swab from holder, press-out excess saline diluent with pressure by rotating against side of tube. Swab each site according to established procedure (see SOP No. 7.2) and return swab to saline tube breaking swab end into tube, and discarding held portion of swab stick.

Feces

Freshly voided fecal samples will be collected in sterile, disposable 4 oz. containers.**

*Sterile Swabs - Falcon Plastics #2078, B-D Laboratories, Baltimore, Maryland.
**Sterile Disposable Sampling Jars - Falcon Plastics #4013, B-D Laboratories, Baltimore, Maryland.

SOP NO. 3.2.2
DATE: 4/16/69
REVISION: 12/22/69

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

3.2 DETECTION PROCEDURES - AEROBIC/ANAEROBIC

3.2.2 ASSAY MEDIA

Primary culturing will be performed using the following media:*

Trypticase Soy Agar with 5% Defibrinated Sheep Blood

Phenylethyl Alcohol Agar with 5% Defibrinated Sheep Blood

MacConkey Agar

Mannitol Salt Agar

Serum Tellurite Agar

Mycosel Agar

LBS (Lactobacillus Selection) Agar

Chocolate Agar

Mycoplasma Agar with Eaton Agent Enrichment

Each of the above media, with the exception of LBS Agar and Mycoplasma Agar, will be procurred** as needed from Baltimore Biological Laboratories, Baltimore, Maryland, in the form of prepared poured plates. Dehydrated LBS Agar and Mycoplasma Agar Base along with Eaton Agent Enrichment will be obtained from Baltimore Biological Laboratories and prepared as needed according to the specific instructions for each recommended by the supplier.

*Quantitative composition and specific directions for preparation of each medium are detailed in Appendix.

**Supplier for Contract NAS 9-9000.

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES	SOP NO. 3.2.3
3.2 DETECTION PROCEDURES - AEROBIC/ANAEROBIC	DATE: 4/16/69
3.2.3 LABELING	REVISION:

Identification of Parameters Involved

All plates and recording charts will be identified with monkey, site sampled, medium employed, fiscal week, and dilution made by means of the following coding system:

PARAMETER IDENTIFIED	CODE	DESIGNATION
Animal	1 thru 10	Monkey One thru Ten
Site Sampled	A	Feces
	B	Gingiva
	C	Throat
	D	Eye
	E	Groin
Culture Medium Employed	BL	Blood Agar
	PEA	Phenylethyl Alcohol Agar
	MAC	MacConkey Agar
	MS	Mannitol Salt Agar
	ST	Serum Tellurite Agar
	M	Mycosel Agar
	R	LBS Agar (Rogosa's Agar)
	CH	Chocolate Agar
	PPLO	Mycoplasma Agar
Time of Sample	1 thru 52	Fiscal Week
Dilution Cultured	-1 thru -10	Log Dilution Factor

Order of Coding

The order of coding will be as follows: Monkey Number, followed immediately by letter code for site samples and fiscal week with media coding and dilution factor directly beneath fiscal week as per the following example:

1A21
MAC -6

This identifies the samples as being the 10^{-6} dilution from the feces of Animal Number 1.

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

3.2 DETECTION PROCEDURES - AEROBIC/ANAEROBIC

3.2.4 SAMPLE PROCESSING

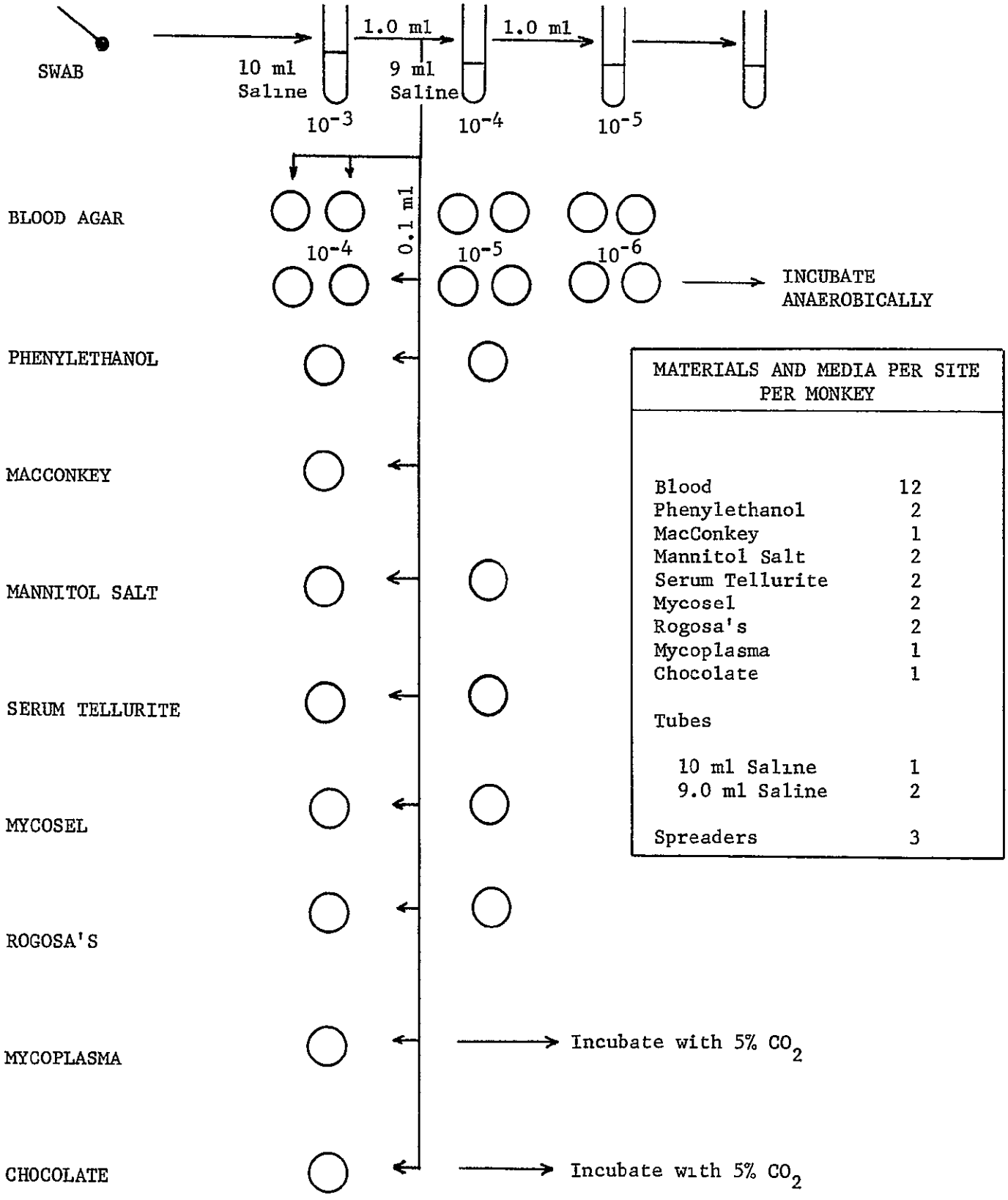
SOP NO. 3.2.4
DATE: 4/16/69
REVISION: 12/22/69

1. Fecal - Processing of each fecal sample will begin as soon as possible after acquisition. Approximately 11 g of the sample (remaining portion is reserved for moisture determination - see SOP No. 3.3) is aseptically transferred to a tared, semimicro monel blending cup, reweighed, and blended for one minute with 99 ml of sterile 0.85% saline at low speed (8,000 rpm). This suspension is arbitrarily designated as a 1:10 (10^{-1}) dilution regardless of exact quantity of feces. Correction for variations in weight of the sample used will be made during calculations after counting has been completed. Ten-fold serial dilutions are made in the 9 ml saline blanks and 0.1 ml aliquots of appropriate dilutions are transferred to agar plates of each designated medium (see Primary Plating Schema for Fecal Sample), and spread evenly over the surface with sterile glass spreaders. For LBS Agar 15-20 ml of the warm, melted medium is poured into sterile petri plates containing 0.1 ml aliquots of the appropriate dilutions. Mixing is accomplished by gently swirling each plate.

2. Swab Samples - Swab samples from remaining four sites will be processed as quickly as possible. If processing cannot proceed promptly, sample tubes should be stored in the refrigerator ($4^{o}C$). Each 10 ml swab tube will be considered as a 1:1000 (10^{-3}) dilution for purposes of quantitation. These tubes will be agitated vigorously on a mechanical vortexer for 10 seconds to insure adequate mixing. One tenth ml aliquots of each appropriate dilution will be transferred to designated agar plates and spread as described previously (see charts of Primary Plating Schema for remaining sample areas).

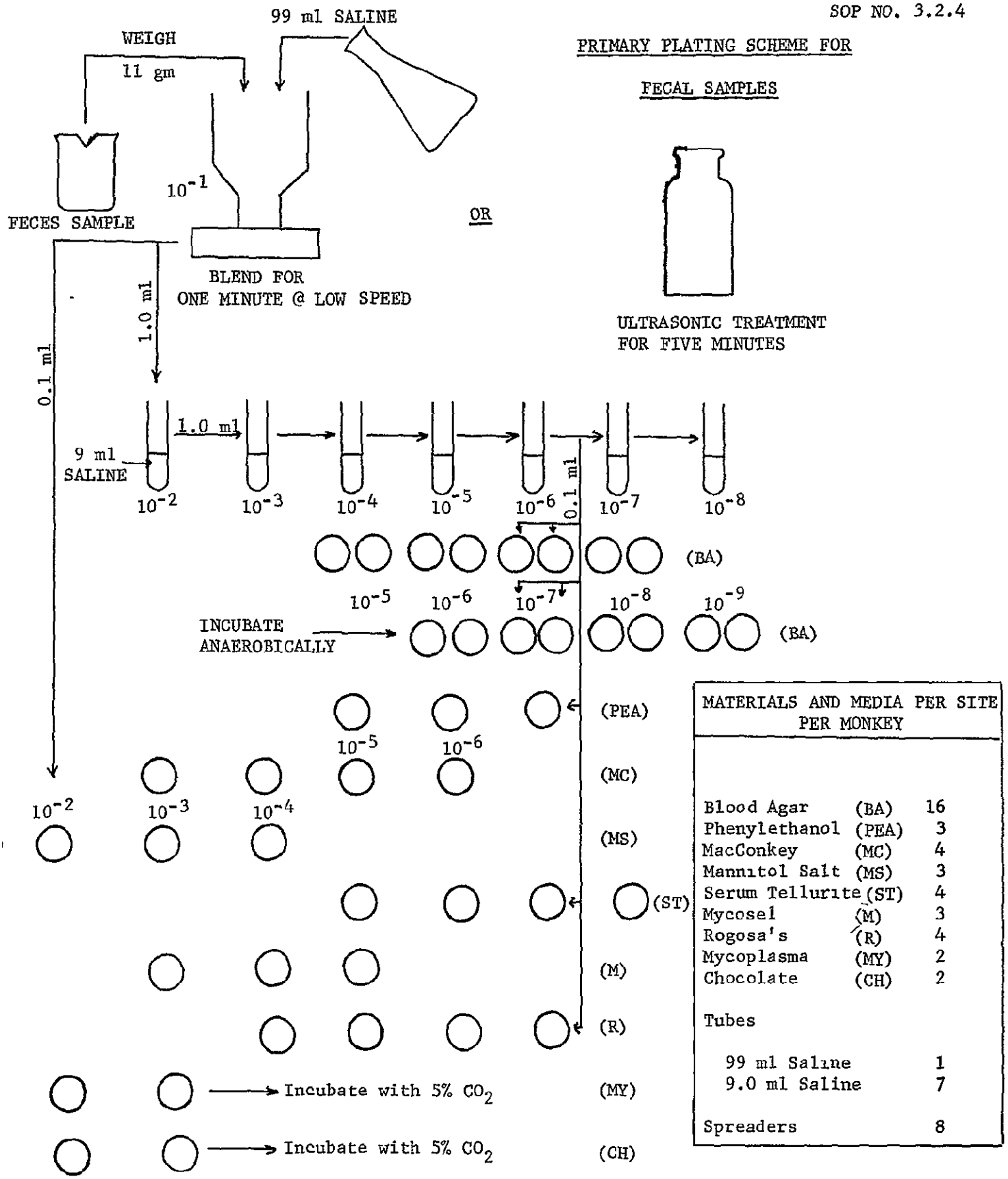
NOTE: Final counts will be recorded as the number of microorganisms per swab; therefore raw counts will be increased 2 logs from number per ml to number per swab.

PRIMARY PLATING SCHEME FOR EYE AND GROIN SAMPLES



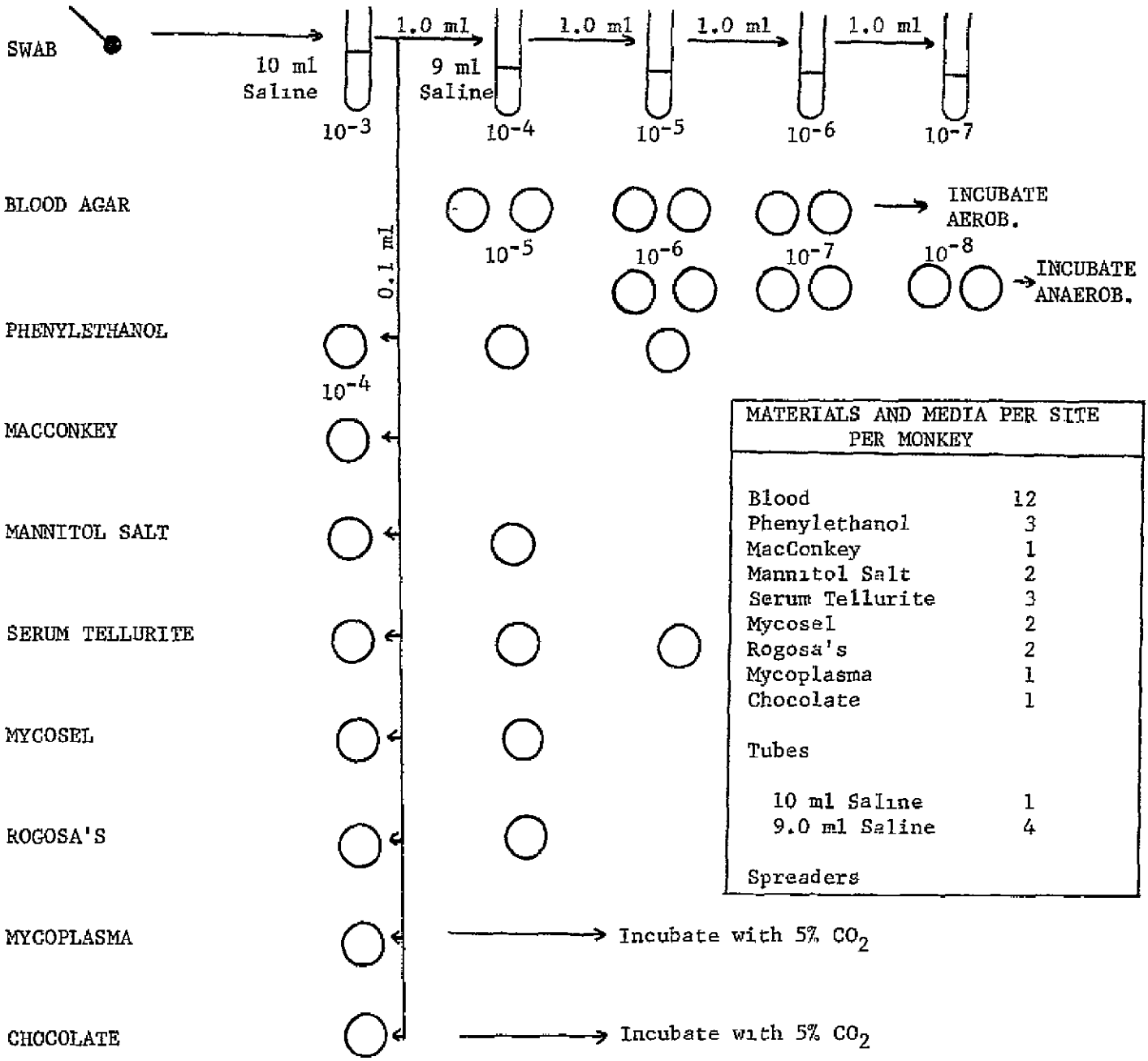
MATERIALS AND MEDIA PER SITE PER MONKEY	
Blood	12
Phenylethanol	2
MacConkey	1
Mannitol Salt	2
Serum Tellurite	2
Mycosel	2
Rogosa's	2
Mycoplasma	1
Chocolate	1
Tubes	
10 ml Saline	1
9.0 ml Saline	2
Spreaders	3

PRIMARY PLATING SCHEME FOR FECAL SAMPLES



MATERIALS AND MEDIA PER SITE PER MONKEY		
Blood Agar	(BA)	16
Phenylethanol	(PEA)	3
MacConkey	(MC)	4
Mannitol Salt	(MS)	3
Serum Tellurite	(ST)	4
Mycosel	(M)	3
Rogosa's	(R)	4
Mycoplasma	(MY)	2
Chocolate	(CH)	2
Tubes		
99 ml Saline		1
9.0 ml Saline		7
Spreaders		8

PRIMARY PLATING SCHEME FOR GINGIVA AND THROAT SAMPLES



MATERIALS AND MEDIA PER SITE PER MONKEY	
Blood	12
Phenylethanol	3
MacConkey	1
Mannitol Salt	2
Serum Tellurite	3
Mycosel	2
Rogosa's	2
Mycoplasma	1
Chocolate	1
Tubes	
10 ml Saline	1
9.0 ml Saline	4
Spreaders	

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES	SOP NO. 3.2.6
3.2 DETECTION PROCEDURES - AEROBIC/ANAEROBIC	DATE: 4/16/69
3.2.6 RECORD KEEPING	REVISION: 12/22/69

Data obtained on microorganisms recovered from animals by the aerobic and anaerobic detection procedures will be recorded on Primary Culture Data Sheets (Form #1) using one sheet for each site on each animal (see sample sheet).

All data obtained on the morphological, cultural, and biochemical characteristics of the microorganisms isolated from the primary detection culture technique will be recorded on Secondary Culture Data Sheets (Form #2). One sheet will be used for each isolate, acknowledging all the information obtained so that the objectives of Section 3.4 for identifying isolates can be met.

All entries will be made in ink.

Anaerobiosis indicators will be checked and initialed.

Counting of plates will be performed with the aid of a Quebec Colony Counter. Appropriate plates will be selected for enumeration (plates containing between 30 and 300 colonies) or in cases where colonies are extremely large, the highest dilution giving readable results.

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

3.2 DETECTION PROCEDURES - AEROBIC/ANAEROBIC

3.2.5 INCUBATION PROCEDURES

SOP NO. 3.2.5
DATE: 4/16/69
REVISION: 12/22/69

1. Aerobic - The following plates will be incubated under aerobic conditions at 35°C ±0.3°C for:

   48 HOURS

   Aerobic Blood Agar

   Serum Tellurite Agar

   Phenylethanol Agar

   Mannitol Salt Agar

   72 HOURS

   Rogosa's Agar

   18-24 HOURS

   MacConkey Agar

   7 DAYS at 25°C

   Mycosel Agar

2. Anaerobic - Anaerobic blood plates will be incubated in Gaspak Anaerobic Jars with "cold" catalysts. Trypticase Soy Agar plates inoculated with Clostridium sporogenes and Alcaligenes faecalis will be used as indicators for anaerobiosis. The cold catalysts will be replaced after five usages. Incubation will be at 35°C ±0.3°C for 48 hours.

3. 3% CO_2 Atmosphere - Chocolate Agar plates and Mycoplasma Agar plates will be incubated in a candle jar with 3-5% CO_2 obtained with the lighted candle technique. Incubation temperature will be 35°C ±0.3°C for 3 days for Chocolate Agar Plates and for 14 days for Mycoplasma Agar plates.

FORM #1

ANIMAL NUMBER _____

SITE: _____

DATE SAMPLED· _____ FISCAL WEEK· _____

PRIMARY CULTURING RESULTS

Log Dıln.	Blood Agar-35°C-48 Hrs.			
	Aerobıc		Anaerobıc	
Total Count				
Adjusted Total Count (Fecal Only)	_____		_____	
Anaerobıc Indıcator	_____			

Fecal Sample Only

Feces + Monel	GM	% Solids	
Tare Alone		Wet Wt.	
Feces Wt.	GM	Dry Wt.	

Dılution Factor = $\frac{\text{Wt. Feces}}{99+\text{Wt.Feces}}$ = _____

$\frac{\text{Dry Wt}}{\text{Wet Wt.}} \times 100 =$ _____ %

$\frac{(\text{Dıln. Factor})(\text{Aerobıc Raw Count})}{\text{\% Solıds}} =$ _____ Vıable Organısms Per Dry Gram

$\frac{(\text{Dıln. Factor})(\text{Anaerobıc Raw Count})}{\text{\% Solıds}} =$ _____ Vıable Organısms Per Dry Gram

PRIMARY SELECTIVE MEDIA

MacConkey - 35°C - 24 Hours			
	Brick Red	Color-less	
Count/Dıln.			
Adj. Count (Fecal)	____	____	____

Mannıtol Salt-35°C-48Hr.			
	Yellow Zone	Pınk	
	____	____	____

Serum Tellurıte Agar-35°C-48 Hr				
	1 mm Black	2-3mm Slate	G/W	
	____	____	____	____

Phenylethanol Agar - 35°C - 48 Hr		
Count/Dıln.		
Adj. Count (Fecal)	____	____

Rogosa's Agar-35°C-72 Hr.			
	Spın-dle	Fuzzy	
	____	____	____

Mycosel Agar - 30°C - 7 Days		
	____	____

Chocolate - 35°C - 48 Hr.			
Count/Dıln.			
Adj. Count (Fecal)	____	____	____

	____	____	____

Pleuropneumonia-Lıke Organısms	
____	

FORM #2

SECONDARY CULTURING DATA

ANIMAL NO. ____________________

SITE ________________________

DATE SAMPLED ______________ FISCAL WEEK __________

SOLATE NO.	COLONY SOURCE AND DESCRIPTION	GRAM STAIN	MOTILITY	CATALASE	OXIDASE	O-F TEST	

SOLATE NO.	TSI			UREA		INDOLE	V-P	$NO_3 \rightarrow NO_2$	GELATIN LIQUIF.	ONPG	MR			Dextrose	Lactose	Maltose	Sucrose	Mannitol	Salicin	Adonitol	Arabinose	Cellobiose	Dextrin	Dulcitol	Galactose	Inositol	Inulin	Melibiose	Raffinose	Rhamnose	Sorbitol	Trehalose	Xylose	
	SLANT	BUTT	H_2S	SLANT	BUTT																													

SAMPLE SHEET

FORM #1

ANIMAL NUMBER: 16

SITE: A (Feces)

DATE SAMPLED: 3/31/69 FISCAL WEEK 14

PRIMARY CULTURING RESULTS

Log Dilu.	Blood Agar-35°C-48 Hrs. Aerobic		Anaerobic	
-7	170	190	140	180
-8	20	15	14	16
Total Count	13×10^9		16×10^9	
Adjusted Total Count (Fecal Only)	54×10^9		4.8×10^9	
Anaerobic Indicator	- K Rod			

Fecal Sample Only

Feces + Monel	5.8510 GM	% Solids	
Tare Alone	5.740	Wet Wt.	1.5C
Feces Wt.	110 GM	Dry Wt.	.5C

Dilution Factor = $\frac{\text{Wt Feces}}{99+\text{Wt. Feces}} = \frac{1 \times 10^1}{}$ $\frac{\text{Dry Wt}}{\text{Wet Wt}} \times 100 = 33\%$

$\frac{(\text{Diln. Factor})(\text{Aerobic Raw Count})}{\% \text{ Solids}} = 54 \times 10^9$ Viable Organisms Per Dry Gram

$\frac{(\text{Diln. Factor})(\text{PM Aerobic Raw Count})}{\% \text{ Solids}} = 4.8 \times 10^9$ Viable Organisms Per Dry Gram

PRIMARY SELECTIVE MEDIA

MacConkey - 35°C - 24 Hours

	Brick Red	Color-less	
Count/Diln.	25 / -5		
Adj. Count (Fecal)			

Mannitol Salt-35°C-48Hr.

	Yellow Zone	Pink	
Count/Diln.	30 / -2	10 / 2	
Adj. Count (Fecal)			

Serum Tellurite Agar-35°C-48 Hr.

	1 mm Black	2-3mm Slate	G/W	
Count/Diln.	70 / -6		20 / -6	
Adj. Count (Fecal)				

Phenylethanol Agar - 35°C - 48 Hr

	Gray 1mm	Rough Sky 1mm	
Count/Diln.	80 / -6	70 / -6	
Adj. Count (Fecal)			

Rogosa's Agar-35°C-72 Hr

	Spin-dle	Fuzzy	
Count/Diln.	10 / -3		
Adj. Count (Fecal)			

Mycosel Agar - 30°C - 7 Days

	Mycelium	Mucoid 5mm	
Count/Diln.	5 / -2	10 / -3	
Adj. Count (Fecal)			

Chocolate - 35°C - 48 Hr

	Glisty Rough		
Count/Diln.	25 / -2		
Adj Count (Fecal)			

Pleuropneumonia-Like Organisms

0 / -2	

SAMPLE SHEET

FORM #2

SECONDARY CULTURING DATA

ANIMAL NO. 16

SITE A (Feces)

DATE SAMPLED 3/31/69 FISCAL WEEK 14

ISOLATE NO.	COLONY SOURCE AND DESCRIPTION	GRAM STAIN	MOTILITY	CATALASE	OXIDASE	O-F TEST	GROWTH AT 41°C	HIPPURATE HYDROLYSIS	
1	MacConkey - dark red spot Bac	G- short rods	+		-	F			
2	MS yellow-tinged	G+ cocci, branches	-	+		F			
3	MS - pink	G+ cocci, branches	-	+		F			
4	ST - 1mm black covered shiny	G+ rods, pleomorphic, uneven staining gram reading effect	-	+		F			
5	ST - gray matte	G+ rods, pleomorphic, uneven staining short forms	-	+		F			
6	FEA - gray, convex, 1mm hemolytic	G+ cocci, short chains	-	-		F	-	+	
7	FEA - 1mm rough dry officials to pink	G+ cocci, short chains	-	-		F	+	-	
8	R - spindle shaped	G+ rods, long twisting chains		-			+		
9	CH - 1mm gray moist	G- cocci, pairs			+				
10	M -								
11									

ISOLATE NO.	TSI			UREA		INDOLE	V-P	$NO_3 \rightarrow NO_2$	GELATIN LIQUIF.	ONPG	MR	COAGULASE TEST	CITRATE UTIL	Dextrose	Lactose	Maltose	Sucrose	Mannitol	Salicin	Adonitol	Arabinose	Cellobiose	Dextrin	Dulcitol	Galactose	Inositol	Inulin	Melibiose	Raffinose	Rhamnose	Sorbitol	Trehalose	Xylose	
	SLANT	BUTT	H_2S	SLANT	BUTT																													
1	Y	Y6	-			+	-				+		-																					Escherichia coli
2												+																						Staphylococcus albus
3												-																						Staphylococcus epidermidis
4								+						+	-	+	-														-			Corynebacterium diphtheriae var mitis
5								+						+	-	+	+														-			Corynebacterium xerosis
6																+		-	-												-	+		Streptococcus agalactiae
7																+		-	+												-	-		Streptococcus mitis
8														+	+	+				+	-		+			+	+				-			Lactobacillus fermenti

SOP NO. 3.4.3
DATE: 4/16/69
REVISION: 12/22/69

SCHEMA FOR PRESUMPTIVE SPECIES IDENTIFICATION

GRAM - NEGATIVE BACTERIA*

POSSIBLE ORGANISM	IDENTIFYING REACTION TO					
	SHAPE	MOTILITY	GROWTH IN AIR	CATALASE	OXIDASE	O-F TEST
Neisseria	S	-	+	+	+	O/-
Gemella	S	-	+	-	-	F
Enterobacteriaceae	R	+/-	+	+/-	-	F
Actinobacillus	R	-	+	+/-	+/-	F
Pasteurella	R	+/-	+	+	+/-	F
Aeromonas	R	+/-	+	+	+	F
Vibrio	R	+	+	+	+	F
Pseudomonas	R	+	+	+	+	O
Chromobacterium	R	+	+	+	-	F/O
Flavobacterium	R	-	+	+	+	O
Acinetobacter	R	-	+	+	-	O/-
Brucella	R	-	+	+	+/-	-
Alcaligenes	R	+	+	+	+	-
Bordetella	R	-	+	NT	d	NT
Haemophilus	R	-	+	NT	d	NT
Bacteroides	R	d	-	d	-	NT
Moraxella	R	-	+	+	+	-

KEY

+ = 80-100% Strains Positive
d = 21-79% Strains Positive
- = 0-20% Strains Positive
F = Fermentation
O = Oxidation
NT = Not Testable
S = Sphere
R = Rod

*Table adapted from Cowan & Steel, 1966, pp. 76-82.

SCHEMA FOR PRESUMPTIVE SPECIES IDENTIFICATION - GRAM - POSITIVE BACTERIA*

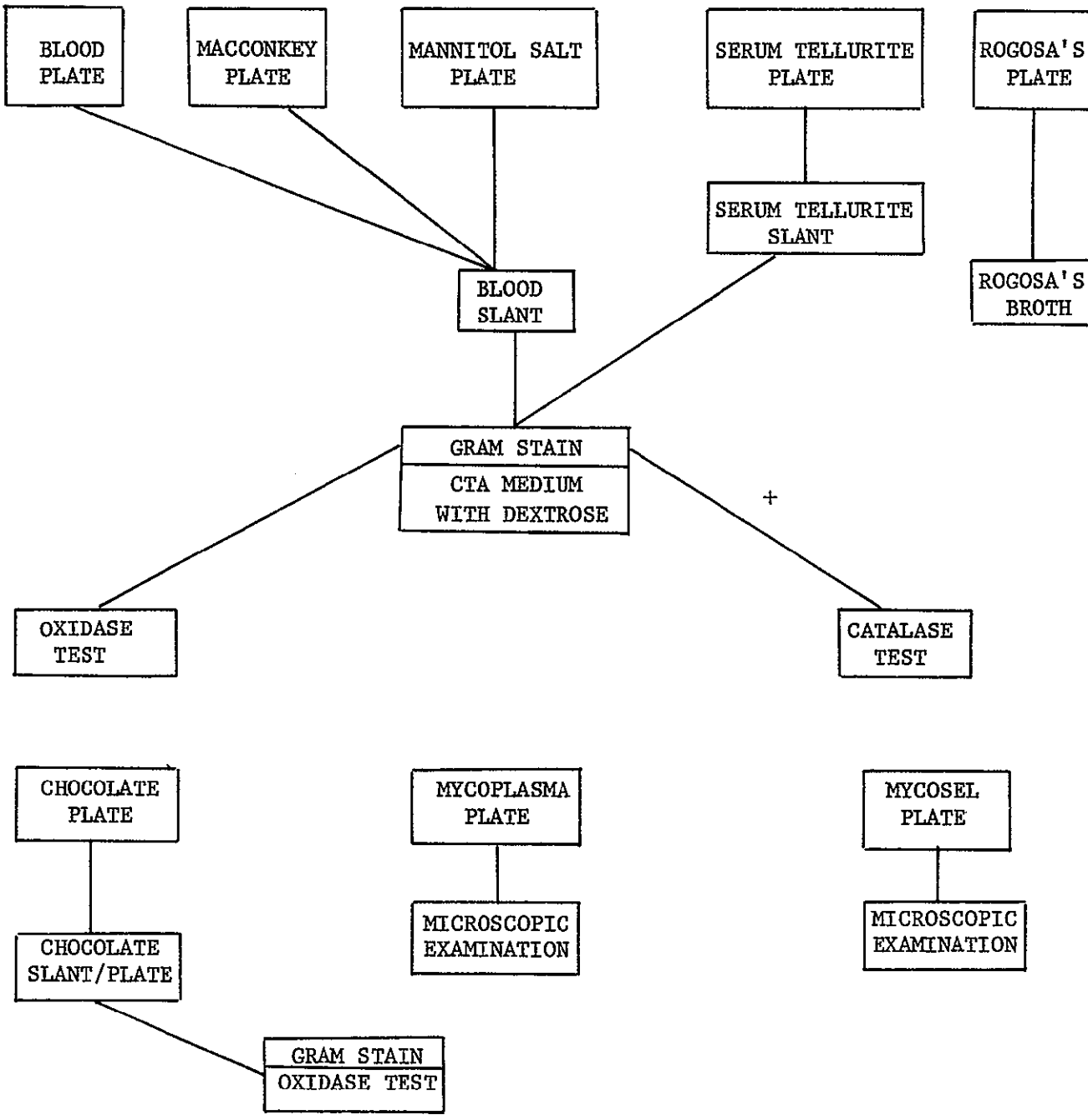
POSSIBLE ORGANISM	IDENTIFYING REACTION TO					
	SHAPE	MOTILITY	GROWTH IN AIR	CATALASE	OXIDASE	O-F TEST
Micrococcus	S	-	+	+	-	O/-
Staphylococcus	S	-	+	+	-	F
Aerococcus	S	-	+	+/-	-	F
Streptococcus	S	+/-	+	-	-	F
Listeria	R	+	+	+	-	F
Corynebacterium	R	-	+	+	-	F/-
Kurthia	R	+	+	+	-	-
Erysipelothrix	R	-	d	-	-	F
Lactobacillus	R	-	d	-	-	F
Actinomyces	R	-	d	-	-	F
Bacillus	R	d	+	+	d	F/O/-
Clostridium	R	d	-	-	-	F/-
Mycobacterium	R	-	+	d	-	O/NT
Nocardia	R	-	+	+/d	-	O/NT

KEY

+ = 80-100% Strains Positive
d = 21-79% Strains Positive
- = 0-20% Strains Positive
F = Fermentation
O = Oxidation
NT = Not Testable
S = Sphere
R = Rod

*Table Adapted from Cowan and Steel, 1966, pp. 55-60.

SCHEMATIC FOR BACTERIOLOGICAL DETECTION PROCEDURES - AEROBIC



3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

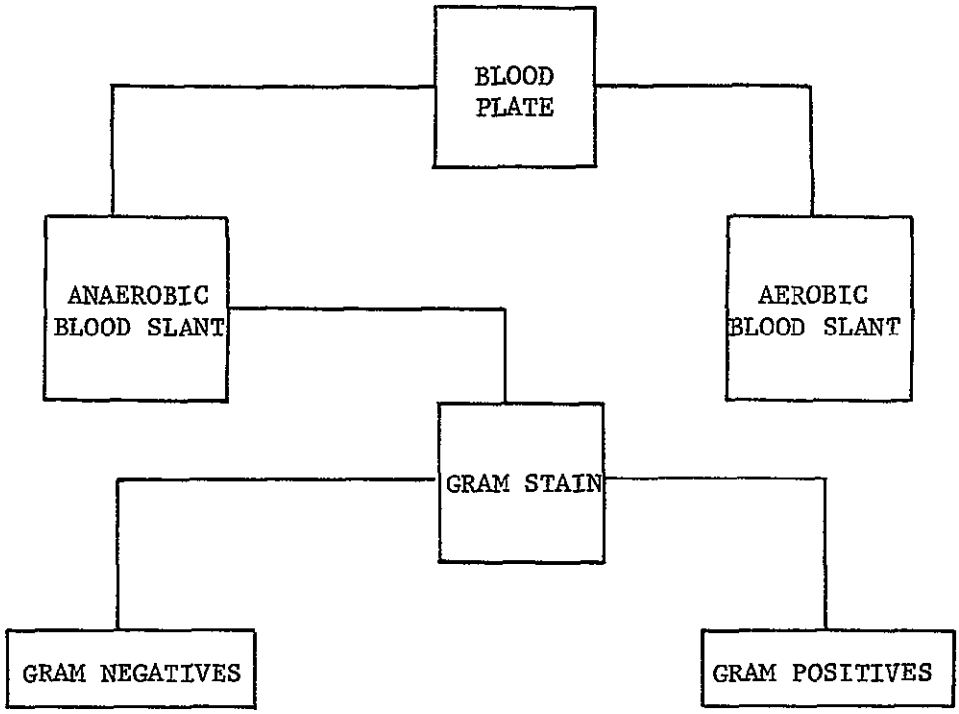
3.4 SECONDARY TESTING - IDENTIFICATION OF ISOLATES

3.4.2 GRAM STAINING/EXAMINATION

SOP NO. 3.4.2
DATE: 4/16/69
REVISION: 12/22/69

SCHEMATIC FOR BACTERIOLOGICAL DETECTION PROCEDURES -

ANAEROBIC



SPHEROPHORUS
BACTERIODES
FUSOBACTERIUM
DIALISTER

PEPTOSTREPTOCOCCUS
ACTINOMTCES
CORYNEBACTERIUM

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

3.4 SECONDARY TESTING - IDENTIFICATION OF ISOLATES

3.4.1 ISOLATION/PURE CULTURE PROCEDURES

SOP NO. 3.4.1
DATE: 4/16/69
REVISION: 12/22/69

Isolated colonies from anaerobic and aerobic cultured agar samples (SOP No. 3.4.2) will be streaked on their appropriate culture medium to obtain a pure culture. These cultures will be retained for further testing as follows:

REMOVED FROM	STREAKED ON
Blood Agar 1. Aerobic 2. Anaerobic	1. Blood Agar Slant 2. Blood Agar Slant
MacConkey Agar	1. Blood Agar Slant
Mannitol Salt Agar	1. Blood Agar Slant
Serum Tellurite Agar	1. Serum Tellurite Agar
Rogosa's Agar	1. Rogosa's Broth
Chocolate Agar	1. Chocolate Slant
Mycoplasma Agar	Microscopic Examination Only
Mycosel Agar	1. Sabourauds Dextrose Agar 2. Microscopic Examination

Anaerobic cultures will be streaked and incubated under anaerobic condition and aerobic condition to insure that they are truly anaerobic organisms.

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

3.4 SECONDARY TESTING - IDENTIFICATION OF ISOLATES

SOP NO. 3.4
DATE: 4/16/69
REVISION: 12/22/69

The Standing Operating Procedures in this sub-section have been generated to satisfy the objective for isolating into pure culture and for identifying the bacteria recovered from the test animals down to the genus level.

3. BASELINE BACTERIOLOGICAL DETERMINATION PROCEDURES

SOP NO. 3.3
DATE: 4/16/69
REVISION: 12/22/69

3.3 FECES MOISTURE DETERMINATION

Freshly voided fecal samples will be collected in sterile, disposable 4 oz. containers.* A 1-2 gram portion is transferred to a disposable aluminum weight pan (Fisher, diameter 4-3/4" x 3/16"). The sample is then placed on a moisture balance (Ohaus). A reading is made and recorded (wet weight). The heating unit is activated and run for 15 minutes. At the end of the time period, the sample is reweighed to obtain the "dry" weight. These weights are recorded in the upper right hand corner of the Primary Culturing Results Data Sheet (Form #1, SOP No. 3.2.6). From these values, the percent of solids is calculated.

*Falcon Plastics #4013

Records will be kept in accordance with SOP No. 10.1.

Information will be kept in tabular form as much as possible to facilitate ease of examination. This Secondary Culture Data Sheet (Form #2, SOP No. 3.2.6) will enable the user to observe the consolidated data.

4. WEEKLY/ROUTINE BACTERIOLOGICAL PROCEDURES

SOP NO. 4 to 4.2.6
DATE: 4/16/69
REVISION· 12/22/69

4.1 GENERAL

The bacteriological testing for weekly operations will all carry the fiscal week number (i.e., FW 23) to indicate the exact time period of the testing. The numbering of samples is detailed in SOP No. 3.2.3. All isolator (test) monkeys and two (2) of the four (4) control monkeys (alternating weeks), will be tested for their individual microbial populations. Swab samples of the gingiva, throat, eyes, and groin, as well as fecal samples will be processed according to SOP No. 3.2.4.

4.2 MONITORING OF "MARKER" ORGANISMS - AEROBIC/ANAEROBIC

1. Monkeys will be kept in close physical proximity prior to being confined in the isolator to establish a common microflora.
2. Animals (control and isolator) were sampled as per SOP's No. 7.2.3 and 7.2.4.
3. The resultant colonies, anaerobic and aerobic, are systematically screened for the selection of the "marker" organisms. The marker organisms are the colonies that are present in the majority of the monkeys.
4. Differentiation of these organisms is done by biochemical testing.
5. The colonies are then catalogued by visual observation. Colonies with specific morphology will be selected.
6. Colonies will be traced weekly in the test animals and bi-weekly with the control animals.

7. No further extensive biochemical test will be carried out unless the marker organisms are lost or reduced to an unreliable population.

8. These procedures will be followed as a guide line and modification for improvement will be made in the discussion of the experimentations.

4.2.1 SAMPLING SITES

The gingive, throat, eyes, and groin will be sampled according to SOP No. 3.2.1 and 7.2. Sampling sites may be altered at the discretion of the experimenters.

4.2.2 ASSAY MEDIA

Assay media will be the same medium used in testing in SOP No. 3.2.2.

4.2.3 LABELING

Labeling will be carried out according to SOP No. 3.2.3.

4.2.4 SAMPLE PROCESSING

Sample processing will be done as in Section 3.2.4 of the SOP.

4.2.5 SAMPLE INCUBATION PROCEDURES

Sample incubation procedures will be handled under the same or similar* conditions in SOP No. 3.2.5.

4.2.6 RECORD KEEPING

All information regarding identification of sampling site(s), organisms recovered, etc. shall be recorded on the appropriate form(s) as identified in SOP Numbers 10.1, 10.3 and 3.2.6.

*With the approval of Laboratory Supervisor, changes to be noted in Journal.

4. WEEKLY/ROUTINE BACTERIOLOGICAL PROCEDURES

SOP NO. 4.3
DATE: 4/16/69
REVISION: 12/22/69

4.3 FECES MOISTURE DETERMINATION

Refer to SOP No. 3.3

5. CARE AND HANDLING OF PRIMATES

SOP NO. 5.1
DATE: 4/16/69
REVISION: 12/22/69

5.1 GENERAL PRACTICES

The isolator and control monkeys will be fed and watered at the same time each day. All animals will receive the same ration each day. The cages and isolators will be cleaned weekly, replenished with new sterile supplies and sterilized (SOP No. 9.2.4). Records will be kept concerning all functions performed on or in the isolator. Major concern must be taken when special situations arise, (leaks, loss of pressure, or violations of sterility).

5. CARE AND HANDLING OF PRIMATES

5.2 ISOLATED PRIMATES

SOP NO. 5.2.1 to 5.2.3
DATE: 4/16/69
REVISION: 12/22/69

5.2.1 FEEDING AND WATERING SCHEDULES

The monkeys will receive a pre-determined diet that has been sterilized by moist heat (SOP No. 8.1). The water to be consumed will be sterilized by procedures in SOP No. 8.2. Each monkey will receive the same number of food pellets and as much water as he desires. The animals will be fed once daily or throughout the day if there is evidence of food waste. Supplementary sterile vitamins (SOP No. 8.3) will be added to each pellet of food. The amount used will be added to each pellet of food. The amount used will be sufficient to overcome the vitamin loss due to sterilization.

5.2.2 CAGE CLEANING

The cages and isolators will be cleaned weekly. The monkeys will be transferred to the small isolator (pass thru) before opening the large flexible isolator.

1. Remove one end of the isolator cover that retains the zipper closures (SOP No. 9.2.2).
2. The inlet and outlet filter openings are sealed with a sterile 6½ rubber stopper.
3. Open the isolator by unzipping the outer fabric zipper first and then the inner plastic zipper.
4. Tighten the support straps to hold up the lower portion of the isolator.

5. Remove the plastic shelf, its' contents, the screened fecal collecting tray, and the urine collection syste. These are then washed with the suitable hot water and soap.

6. The isolator and cage are washed with an appropriate hot water and soap, and rinsed thoroughly with spray nozzles from a water hose. Drain tubes are attached to the bottom of the isolators drain plugs to remove the rinse water. Each isolator at a time has a cage change weekly so that all four cages are changed each month.

7. Replace the items removed from the isolator and add new supplies (SOP No. 9.3.6).

8. Close the isolator using SOP No. 9.2.2.

9. Spray with 2% Peracetic Acid solution (SOP No. 9.2.4) to sterilize the isolator. All items, cage and surfaces of the isolator -- exposure time one hour.

10. Flush the isolator with sterile air for 20 hours (approximately 12:00 PM to next morning 8:00 AM) to remove residual peracetic acid solution. The isolator may have to be wiped dry with sterile rags or drained to remove excess moisture and peracetic acid before sufficient flushing occurs (air-peracetic acid).

5.2.3 RECORD KEEPING

Records will be kept according to SOP Nos. 10.1 and 10.2 with special emphasis placed on irregular or re-occurring malfunctions in equipment.

5. CARE AND HANDLING OF PRIMATES

5.3 CONTROL (NON-ISOLATED) PRIMATES

SOP NO. 5.3.1 to 5.3.3
DATE: 4/16/69
REVISION: 12/22/69

5.3.1 FEEDING AND WATERING PROCEDURES

The control monkey will receive the same food and water as the isolated monkey. They will be handled in the same manner as SOP No. 5.2.1.

5.3.2 CAGE CLEANING

The control monkeys will have their cages sprayed down daily with one clean cage change per animal per week. The soiled cages will be soaked with scrubbing in a suitable detergent and rinsed with tap water from a spray hose. These cages are not sterilized, but are periodically steam cleaned with hot flowing steam.

5.3.3 RECORD KEEPING

Records will be kept in the same manner as SOP No. 10.1. Control monkeys will not be assigned a specific cage due to the frequent cage changes, but data books will be kept on each monkey.

6. SEROLOGICAL PROCEDURES

SOP NO. 6.1
DATE: 4/16/69
REVISION: 12/22/69

6.1 INTRODUCTION

The Standing Operating Procedures to determine the major serological parameters are included in this section. The objective of the serological determinations was to detect only drastic alterations in the animals immune character affected by the conditions of the isolation experiment.

6. SEROLOGICAL PROCEDURES

6.2 TESTS REQUIRED/FREQUENCY

SOP NO. 6.2
DATE: 4/16/69
REVISION: 12/22/69

The serological tests performed on the primates include:

White Blood Cell Total Count

Red Blood Cell Total Count

Differential Leukocyte Count

Platelet Estimation

Hemocrit Determination

Hemoglobin Determination

RBC Indices (MCV, MCH, MCHC)

Serum Analysis

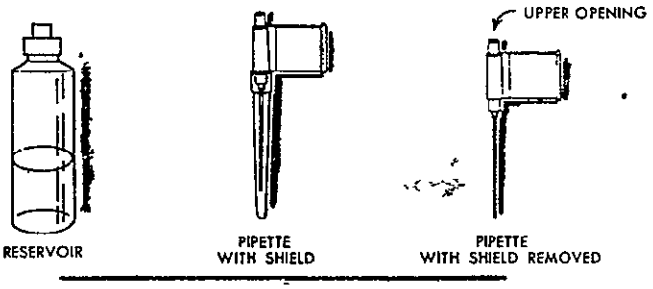
The frequency of these tests correspond with the bi-weekly microbial determinations.

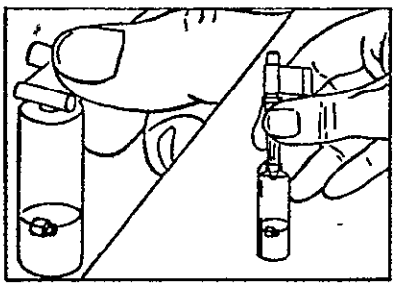
6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

SOP NO. 6.3
DATE: 7/22/69
REVISION : 12/22/69

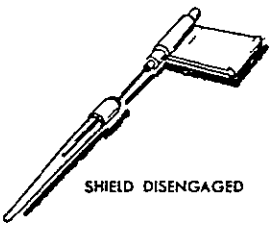
UNOPETTE COLLECTION AND DILUTION TECHNIQUE

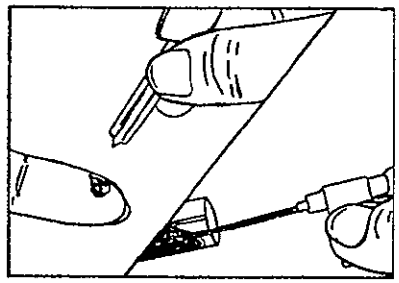




(1) Using capillary flag as shown, apply firm, even pressure and depress plug on top of plastic reservoir Use tip of capillary shield, if necessary, to drop plug into reservoir, where it will serve as the mixing "bead"

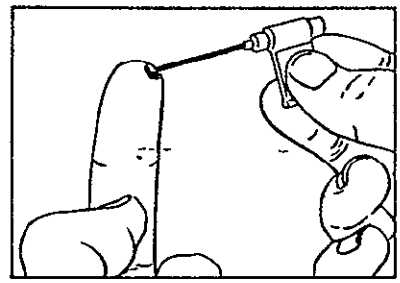
(2) Disengage shield from one of the pipettes with a twist action and leave the pipette loose in the shield (ds shown) until ready to use.

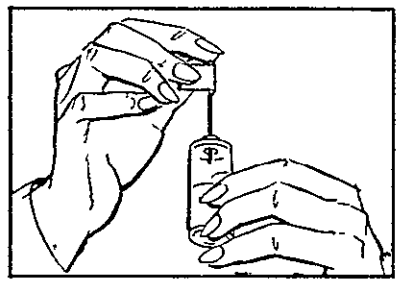




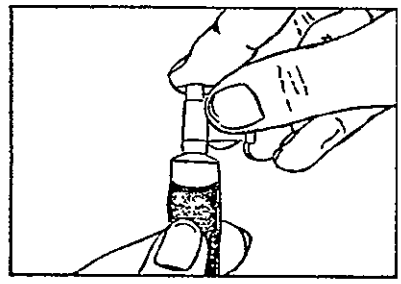
(3) Obtain blood sample from free flowing finger puncture (or use thoroughly mixed venous blood specimen)

(4) Holding capillary almost horizontally, touch tip to blood as shown Capillary action fills tube and blood collection stops automatically—avoiding error inherent in drawing blood in conventional pipettes Wipe any blood off outside of capillary, making sure no blood is removed from capillary bore



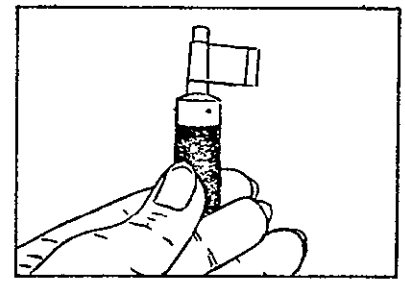


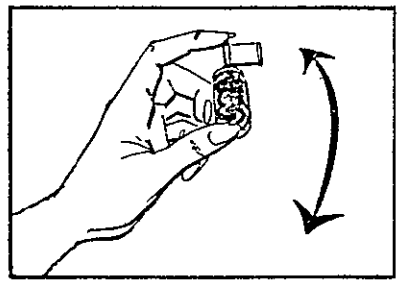
(5) Insert pipette capillary into reservoir



(6) Squeeze reservoir slightly, cover upper opening of pipette with index finger and seat holder in reservoir neck Release pressure on reservoir and remove finger from pipette opening Suction will draw blood into diluent

(7) Squeeze reservoir gently two or three times to rinse capillary tube, forcing diluent up into — but not out of — overflow chamber, releasing pressure each time to return rinsing diluent to reservoir Exact blood dilution has now been made automatically with pre-measured diluent





(8) Place index finger over upper opening of pipette and gently invert a few times to thoroughly mix blood and diluent

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.1 WBC TOTAL COUNT

SOP NO. 6.3.1
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To determine the number of leukocytes (white blood cells) in the blood.

MATERIALS

Unopette reservoir containing 1.3 ml 1% ammonium oxalate

13 µl micro-pipette

Hemacytometer and cover glass

Microscope

PROCEDURE

STEP	REMARKS
1. Collect 13 µl blood in a micro-pipette.	1. See Unopette collection and dilution instructions. (SOP #6.
2. Insert pipette into reservoir containing 1.3 ml 1% ammonium oxalate.	
3. Draw blood into reservoir and mix with diluent.	3. See Unopette collection and dilution instructions. (SOP #6.
4. Remove pipette from reservoir.	
5. Invert and place finger on capillary tip.	
6. Compress reservoir.	
7. Keeping finger on capillary tip, seat pipette in reservoir.	
8. Release compression on reservoir and uncover tip of capillary.	8. Any fluid in the capillary will be drawn into the reservoir.
9. Tilt and squeeze reservoir to release drops of diluted blood.	9. The dilution ratio is 1:100 inst of the 1:20 normally used with conventional methods.

PROCEDURE (Continued)

STEP	REMARKS
10. Charge hemacytometer by placing the capillary tip on the floor of the basin in back of the edge of the cover glass.	
11. Gently release a drop of liquid and remove the pipette as soon as the liquid reaches the level of the cover glass and starts its capillary influx.	11. The liquid should amply cover the ruling or fill the entire chamber without overflowing into the surrounding moats. If not, repeat the charging operation after the chamber has been cleane with water.
12. Do not adjust the cover glass nor in any way disturb the chamber after it has been filled.	
13. Wait approximately 3 minutes for settling before removing hemacytometer to the microscope stage.	
14. Locate the ruled area of the chamber with the low power objective.	
15. Count the total number of leukocytes in all 9 squares of the chamber grid.	15. This allows for the greater dilution of blood.
16. Add 10% to the total number of cells counted and multiply by 100 to obtain cell count per cu. mm.	

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.2 RBC TOTAL COUNT

SOP NO. 6.3.2
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To determine the number of erythrocytes (red blood cells) in the blood.

MATERIALS

Unopette reservoir containing 2.6 ml isotonic saline.

13 µl micro-pipette

Hemacytometer and cover glass

Microscope

PROCEDURE

STEP	REMARKS
1. Collect 13 µl blood in a micro-pipette.	1. See Unopette collection and dilution instructions. (SOP #6.3)
2. Insert pipette into reservoir containing 2.6 ml isotonic saline.	
3. Draw blood into reservoir and mix with diluent.	3. See Unopette collection and dilution instructions. (SOP #6.3)
4. Remove pipette from reservoir.	
5. Invert and place finger on capillary tip.	
6. Compress reservoir.	
7. Keeping finger on capillary tip, seat pipette in reservoir.	
8. Release compression on reservoir and uncover tip of capillary	8. Any fluid in the capillary will be drawn into the reservoir.
9. Tilt and squeeze reservoir to release drops of diluted blood.	9. The dilution ratio is 1:200.
10. Charge hemacytometer by placing the capillary tip on the floor of the basin in back of the edge of the cover glass.	

PROCEDURE (Continued)

STEP	REMARKS
11. Gently release a drop of liquid and remove the pipette as soon as the liquid reaches the level of the cover glass and starts its capillary influx.	11. The liquid should amply cover the ruling or fill the entire chamber without overflowing into the surrounding moats. If not, repeat the charging operation after the chamber has been cleaned with water.
12. Do not adjust the cover glass nor in any way disturb the chamber after it has been filled.	
13. Wait approximately 3 minutes for settling before removing hemacytometer to the microscope stage.	
14. Locate the ruled area of the chamber with the low power objective.	
15. The center square is divided into 25 smaller squares. Count the number of erythrocytes in the 4 corner squares and one center square.	15. Use a high dry objective.
16. Total the RBC's in the 5 squares and multiply by 10,000 to obtain cell count per cu. mm. ____	

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.3 DIFFERENTIAL COUNT

SOP NO. 6.3.3
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To differentiate the type and determine the proportion of leukocytes.

MATERIALS

Microscope slides

Microscope

Wright's stain

Distilled Water

PROCEDURE

STEP	REMARKS
1. Use slides which have been cleaned in isopropyl alcohol.	
2. Pick up a small drop of blood on the slide approximately 1/4 inch from the end.	2. The slide should not come in contact with the skin.
3. Place the slide on a flat surface.	
4. Immediately approach the drop of blood with second slide held at a 30° angle.	4. The angle may be changed depending on the size of the blood drop.
5. Allow the blood to run across the edge of the second slide.	
6. Push the blood with the second slide across the surface of the original slide to form a thin blood film.	6. The film should not touch the edge of the slide.
7. Let the film completely dry.	
8. Place the dry slides on a staining rack.	8. For best results, stain the slides within 24 hours.
9. Flood the slide with Wright's stain.	9. No fixative is necessary since Wright's stain contains methyl alcohol.

PROCEDURE (Continued)

STEP	REMARKS
10. Wait three minutes.	
11. Add distilled water until there is an equal volume of stain and water.	11. A greenish film should appear on the surface of the mixture.
12. Wait five minutes.	12. This period may be lengthened if the stain has not aged long enough.
13. Wash the slide with distilled water.	
14. Blot the slide between layers of bibulous paper.	14. The slides may be restained if necessary.
15. Examine the leukocytes using the oil immersion objective.	
16. Count 100 leukocytes differentiating them into the various types.	
17. Take a representative count being careful not to repeat any microscopic field.	
18. Report the leukocytes in percentages.	

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.4 PLATELET ESTIMATION

SOP NO. 6.3.4
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To determine by qualitative estimation the adequate presence of platelets in the blood.

MATERIALS

Wright's stained differential blood smear

Microscope

PROCEDURE

STEP	REMARKS
1. While examining a differential blood smear, ascertain the presence of platelets in each microscopic field.	1. This procedure is a subjective estimation only.
2. If platelets are present in all fields or all but one field, report as "adequate".	
3. If platelets are not present in 2-3 fields, report as "possibly inadequate".	
4. If platelets are not present in more than three fields, report as "inadequate".	

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.5 HEMATOCRIT

SOP NO. 6.3.5
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To measure the volume of packed red blood cells.

MATERIALS

Micro-hematocrit capillary tubes, non-oxalated, 0.8 mm x 32 mm.
Seal-Ease tube sealer.
International micro-capillary centrifuge, Model MB.
International micro-capillary reader (Circular Type I.E. 2201).

PROCEDURE

STEP	REMARKS
1. Fill a capillary tube 2/3's full of blood.	
2. Immediately push the dry end of the tube down thru the Seal-Ease.	
3. Rotate the tube between the fingers.	3. This prevents sealing plug from pulling out upon withdrawal.
4. Remove tube and press the sealed end against a finger tip.	4. This smooths the sealer and prevents leakage.
5. Remove the cover from the centrifuge head.	
6. Place sealed tubes in numbered slots with sealed end at the periphery.	
7. Replace head cover and tighten cover-nut onto the head finger tight. Close cover.	
8. Centrifuge four minutes	8. The proper speed for hematocrits has been pre-set. (Set by manufacturer 11,500 rpm.)
9. Lift cover and unscrew head cover.	
10. Promptly remove tubes and determine the hematocrit by placing each tube in the reader.	
11. Follow the directions printed on the reader to obtain the percent cell volume.	

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.6 HEMOGLOBIN

SOP NO. 6.3.6
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To determine the total hemoglobin content of the blood.

MATERIALS

Unopette reservoir containing 1.3 ml 1% ammonium oxalate

13 µl micro-pipette

Spectrophotometer

Cuvettes

Hycel cyanmethemoglobin reagent (Drabkin's solution)

Hycel cyanmethemoglobin standard

Hycel hemoglobin control

Graph paper

PROCEDURE

STEP	REMARKS
1. Dilute blood using standard Unopette procedure.	1. The hemoglobin determination is made on the same Unopette as the WBC after the leukocytes have been counted.
2. Dispense diluted blood into a cuvette containing 2 ml cyanmethemoglobin reagent by forcing contents thru capillary tube.	
3. Rinse capillary tube and reservoir.	3. Draw the liquid back into the reservoir and redispense into the cuvette.
4. Read in spectrophotometer at a wavelength of 540 mµ against a reagent blank.	4. Use a blank of 3.3 ml cyanmethemoglobin reagent and adjust to zero optical density.

PROCEDURE (Continued)

STEP	REMARKS
5. Obtain hemoglobin concentration in gm % from a standard curve prepared as follows:	5. Use Hycel cyanmethemoglobin standard. Periodically check the standard curve. Hycel hemoglobin control may also be used as a check on the accuracy of the procedure.

	B	5	10	15	20 gm %
Volume of Standard	0	1.5	3.0	4.5	6.0 ml
Volume of Reagent	6.0	4.5	3.0	1.5	0. ml

6. Plot optical density against concentration (gm % hemoglobin) on regular graph paper.

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.7 RBC INDICES

SOP NO. 6.3.7
DATE: 7/22/69
REVISION: 12/22/69

PURPOSE

To express characteristics of red blood cells in terms of size, hemoglobin content and hemoglobin concentration.

MATERIALS

Known values for RBC, hemoglobin and hematocrit

PROCEDURE

STEP	REMARKS
1. MCV (mean corpuscular volume) $MCV = \frac{\text{Hematocrit X 10}}{\text{RBC} \div 1{,}000{,}000}$ The MCV is expressed in cubic microns (cμ)	1. To determine the mean size of red blood cells.
2. MCH (mean corpuscular hemoglobin) $MCH = \frac{\text{Hgb X 10}}{\text{RBC} \div 1{,}000{,}000}$ The MCH is expressed in micro-micrograms (μμg).	2. Indicates the weight of hemoglobin per average red blood cell.
3. MCHC (mean corpuscular hemoglobin concentration) $MCHC = \frac{\text{Hgb}}{\text{Hematocrit}} \text{ X } 100$ The MCHC is expressed in percentage (%)	3. Indicates the concentration of hemoglobi per red blood cell.

6. SEROLOGICAL PROCEDURES

6.3 TEST PROCEDURES

6.3.8 SERUM SAMPLES

SOP NO. 6.3.8
DATE: 4/16/69
REVISION: 12/22/69

Samples of blood from each monkey were removed, centrifuged at 5,000 RPM's to separate the solids from the serum and retained at $4^{o}C$ in sterile injectable bottles for further use in a possible follow-on study.

6. SEROLOGICAL PROCEDURES

6.4 RECORD KEEPING

SOP NO. 6.4
DATE: 4/16/69
REVISION: 12/22/69

All data obtained from the serological and hematological tests will be recorded on the following form (Form #3) for each monkey on the date sampling was performed.

Any deviations from the normal range of values expected for each index will be brought to the immediate attention of the program manager.

HEMATOLOGY DATA

MONKEY NUMBER: _____ DATE: _____

	TEST VALUES	NORMAL VALUES
HGB	_____	11-12.5 gm/100 ml
PCV	_____	39-43%
RBC	_____	5-6 x 10^6/mm^3
WBC	_____	7-13 x 10^3/mm^3
PLAT. EST.	_____	ADEQUATE (350-625 T/mm^3 or x 10^3 cells/mm^3)
DIFF.	_____	
BANDS	_____	5-10%
SEGS.	_____	15-46%
TOTAL NEUTROS	_____	20-56%
LYMPHS	_____	40-76%
MONOS	_____	0.5-2.0%
EOS	_____	1-3%
BASOS	_____	0-2%
RBC INDICES		
MCV	_____	65-78 μ^3
MCH	_____	18-22 $\mu\mu$ gm.
MCHC	_____	27-31 gm/100 ml

7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES

SOP NO. 7.1
DATE: 4/16/69
REVISION: 12/22/69

7.1 GENERAL

All items used in the isolators are sterilized before being used in the isolator (SOP No. 8.4.1). These items are introduced, through the small rigid isolator attached to the larger flexible isolator, with spraying of 2% peracetic acid solution (SOP No. 9.3.5 and 9.2.4) all samples will be removed and asceptic condition with pass-thru (rigid isolator) being re-sprayed after samples are removed.

7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES

7.2 ISOLATED PRIMATES

7.2.1 MATERIALS REQUIRED (SOP No. 9.3.6)

SOP NO. 7.2.1
DATE: 4/16/69
REVISION: 12/22/69

1. Sernyla n* (phencyclidine hydrochloride) injectable
2. 1 cc TB sterile syringe

   2½ cc sterile syringe
3. Sterile 9.0 ml 0.85% saline (SOP No. 8.4.3)

   Sterile 99.0 ml 0.85% saline (SOP No. 8.4.3)
4. Sterile cotton swabs (Swube-Falcon Plastics #2078)
5. Sterile Heparin Sodium 1:500 (SOP No. 8.4.4)
6. Sterile 4 oz. fecal container (Falcon Plastics #4013)
7. Sterile 4 inch stainless steel spatula
8. Sterile spring scale graduated in kg.
9. Sterile 2" x 2" gauze pads
10. Sterile 70% isoproponal (SOP No. 8.4.7)

*Parke-Davis Company, Detroit, Michigan.

7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES

7.2 ISOLATED PRIMATES

7.2.2 PREPARATION OF PRIMATES

SOP NO. 7.2.2
DATE: 4/16/69
REVISION: 12/22/69

1. Monkey is brought to the front of the cage with the "Squeeze" bar.
2. A 0.15 cc injection of Sernylan* (phencyclidine hydrochloride) is prepared and administered to the upper heavy muscle of the hip. If it is impossible to reach this area, the injection is given in the fleshy portion of the shoulder. The animal is immobile in approximately 3-5 minutes.
3. When immobile, he is removed from the cage for sampling.

*Parke-Davis Company, Detroit, Michigan

7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES

7.2 ISOLATED PRIMATES

7.2.3 SAMPLE PROCUREMENT PROCEDURE - BACTERIOLOGICAL

SOP NO. 7.2.3
DATE: 4/16/69
REVISION: 12/22/69

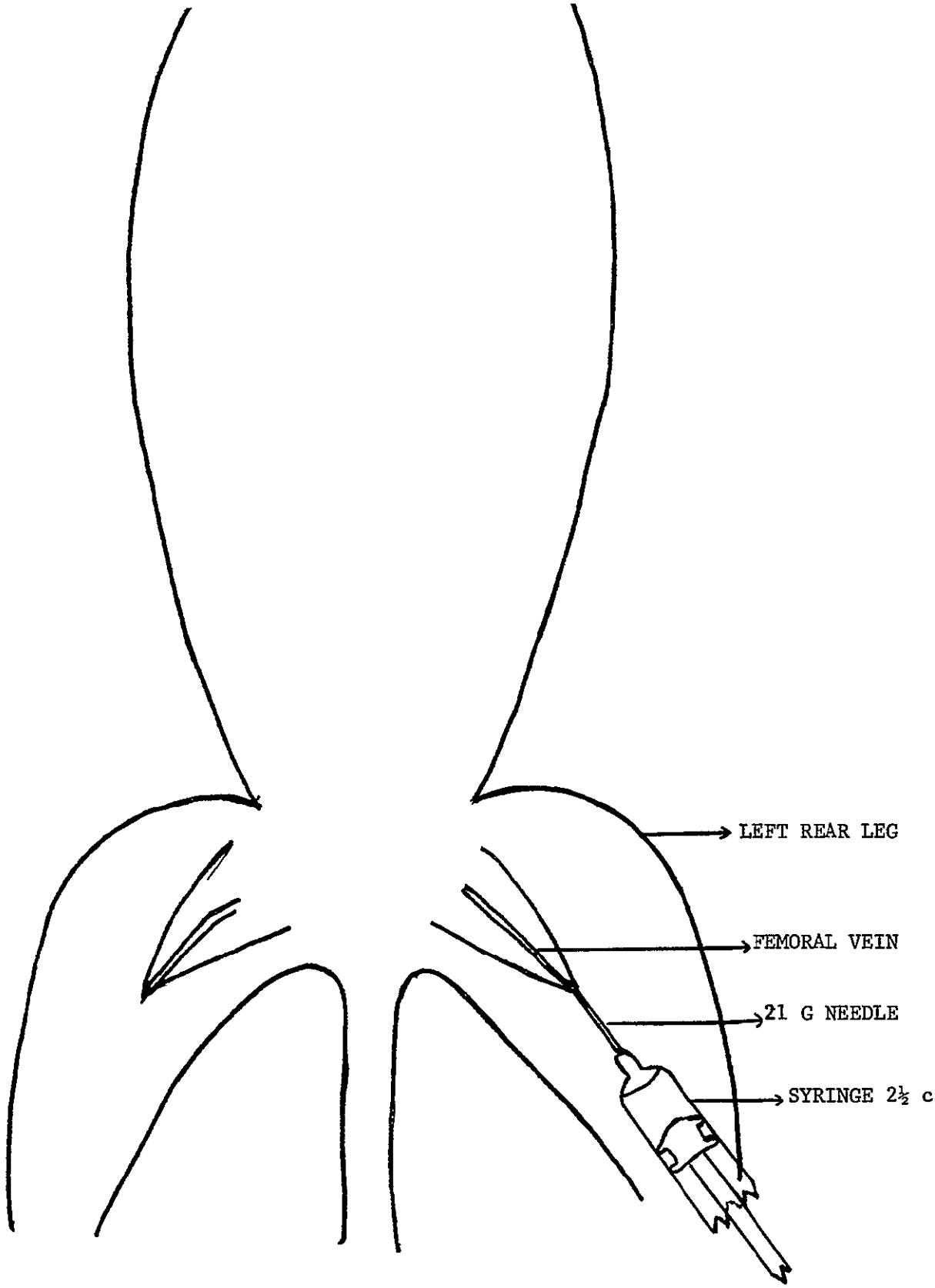
1. Monkey is immobilized by injection of Sernylan* (phencyclidine hydrochloride), SOP No. 7.3.2, and removed from the cage and placed on the isolator work table with his head toward the operator.
2. Place the fingers of the left (right) hand in the outer corners of the mouth exerting inward pressure, this forces open the animal's mouth.
3. Cotton applicator (Swube) is moistened in a 10 ml 0.85% saline blank - press out excess saline diluent with pressure against side of tube. After the sample is taken, it is returned to the tube as in SOP No. 3.2.1.
4. Scrub the gingiva and groin area in a backward and forward motion, then with an up and down motion. The swab preparation is the same for all sampling areas (Step 3). The throat is sampled by opening the mouth as in Step 2. Rotate the swab in a circular motion in the throat covering as much area as possible. Care should be taken not to force the animal to vomit as the resultant product may cause the animal to choke in the immobilized state.
5. The swab is prepared in the same manner as Step 3. The thumb and first finger are placed on either side of the eye to be sampled. The eyelids are held open and the moistened swab is placed on the eye. The eyelids are released allowing the animal to move the swab by blinking the eye.
6. A freshly voided fecal sample will be collected in a 4 oz. container, (Falcon Plastic #4013) using a sterile stainless steel spatula (SOP No. 2.4.3). A large portion of the sample is transferred to a pre-weighed 99 ml saline blank (SOP No. 8.4.3) for use in the microbial evaluation of the feces (SOP No. 3.2.4).

7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES	SOP NO. 7.2.4
7.2 ISOLATED PRIMATES	DATE: 4/16/69
7.2.4 SAMPLE PROCUREMENT PROCEDURE - SEROLOGICAL	REVISION: 12/22/69

1. Animal is immobilized as in Section 1, SOP No. 7.2.3. Animal's head is away from operator.
2. A sterile 2½ cc syringe is rinsed with sterile heprin sodium (1:500 units), SOP No. 8.4.4.
3. The inside of the right or left leg is washed with sterile 70% isopropanol (SOP No. 8.4.7). This is the area for the insertion of the needle. The blood is removed from the femoral vein (see Figure 1) (approximately 0.50 cc) and transferred to a screw cap tube (13 x 100 mm)* (SOP No. 8.4.6). The needle is removed from the syringe before the transfer is made to prevent the clotting and the rupturing of the red blood cells.
4. When the needle is removed, the puncture has a guaze pressure pad applied to prevent the bleeding of the animal. The pad is held tightly until all bleeding is stopped.

*Fisher Scientific Supply, Gulph Road, King of Prussia, Pa., 19406

FIGURE 1



7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES	SOP NO. 7.2.5
7.2 ISOLATED PRIMATES	DATE: 4/16/69
7.2.5 REMOVAL OF SAMPLES	REVISION: 12/22/69

Sealed samples are placed in the small rigid isolator. After the inside door is closed and the air inlet and outlet are closed, the samples are removed for processing. The small isolator is then sprayed with 2% peracetic acid solution and allowed to stand for 30 minutes prior to flushing with air (SOP Numbers 9.2.4 and 9.3.5).

7. BACTERIOLOGICAL AND SEROLOGICAL SAMPLING PROCEDURES

7.3 CONTROL (NON-ISOLATED) PRIMATES

SOP NO. 7.3.1 to 7.3.4
DATE: 4/16/69
REVISION: 12/22/69

7.3.1 MATERIALS REQUIRED

The same materials that are used for the isolator monkeys are used for the control group (SOP No. 7.2.1).

7.3.2 PREPARATION OF MONKEYS

Animals are prepared in the same manner as isolator animals, (SOP No. 7.2.2).

7.3.3 SAMPLE PROCUREMENT PROCEDURE - BACTERIOLOGICAL

The same as SOP No. 7.2.3.

7.3.4 SAMPLE PROCUREMENT PROCEDURE - SEROLOGICAL

The same as SOP No. 7.2.4.

8. PRIMATE DIET PREPARATION

SOP NO. 8.1, 8.1.1, 8.1.2
DATE: 4/18/69
REVISION: 12/21/69

8.1 FOOD

All food supplies for use inside the primate isolators and by control animals will be sterilized by the following procedures.

8.1.1 PREPARATION OF TRANSFER ISOLATOR SYSTEM

1. Sterilize eight large screw cap gallon jars* in the autoclave for 20 minutes at STP employing slow exhaust, 10-15 ml water inside of each jar, and loose fitting lids. Tighten all lids immediately before removing from autoclave.
2. Inside a flexible holding isolator, place the following items:
   (a) Sterile screw cap gallon jars (remove all autoclave tape).
   (b) Test tube rack containing four sterile swabs and four tubes containing 10 ml of Trypticase Soy Broth.
   (c) Stainless steel spatula.
   (d) Spray bottle of 2% peracetic acid prepared according to SOP No. 9.3.5.
   (e) Inside isolator cover with spring or rubber band closure.
   (f) Two No. 3 rubber stoppers to plug air intake/exhaust of isolator.
3. Place outside cap/cover on isolator transfer tunnel and tape in place.
4. Sterilize interior of the isolator with 2% peracetic acid. Be certain all crevices, folds, and surfaces (including inside surface of pass-through) are wetted with the spray. DO NOT open jars. Allow contents to remain static for at least one hour, then place spray bottle in pass-through and replace the inside cap, affix securely with the spring or rubber band.

*Obtained locally

8.1.2 PREPARATION OF FOOD AND STERILITY ASSURANCE

1. Package primate food pellets (Rockland Star-Shape Monkey Chow)* in 42 ATI Sterilıne Bags,** 14 pellets/bag, net weight ıs 8 grams per pellet. Fold each bag wice and seal wıth tape. These 42 bags shall constitute a lot number for record purposes.
2. Place bags on shelf of large sterilizing drum, four abreast. The two bags in the approxımate center of the layer will each contain one "Kilıt"*** ampule, properly labeled for future identification. DO NOT PLACE MORE THAN 42 BAGS IN DRUM.
3. Seal drum with "Mylar" tape and "Scotch" tape and sterilıze for 45 minutes usıng fast exhaust and dry to remove moisture.
4. Using connector sleeve, connect and sterilıze drum to the holding isolator and sterilize sleeve with peracetic acid.
   Allow to remain static for one hour.
5. Remove cap from inside pass-through and rubber stoppers from air intake/exhaust. Allow filtered air to enter system overnight.
6. The following morning, take sterility samples from the followıng areas using the sterile swabs.
   (a) Air ıntake
   (b) Floor of isolator
   (c) Between glove ports
   (d) Aır exhaust
   Break swabs into tubes of Trypticase Soy Broth*** and set to one side.

*Rockland Primate Diet, Dıstributed through TECHLAB, Inc., 55 Public Square, Monmouth, Illinois
**Aseptıc Thermo Indicator Company, through Joseph E. Frankle Co., Lab Supplies and Equıpment, Philadelphia, Pa. (Cat. No. 276)
***Baltımore Biological Laboratory, Division of Becton, Dickson Co., Box 175, Cockeysvılle, Maryland 21030 (Stock No. 12018)

7. Break "Mylar" tape on drum with spatula and transfer food from drum to interior of isolator. Place five bags of food in with jar and reseal jar. Place two bags containing "Kilits" to one side.
8. Replace caps to air intake/exhaust, open system, and remove jars. Label each Trypticase Soy Broth tube and "Kilits" ampules and incubate at 35°C for seven days.
9. Enter results of sterility checks in notebooks under proper food lot number. Between one and two weeks supply of food bags should be on hand at all times to insure proper holding time for sterility checks and in case of emergency.

8. PRIMATE DIET PREPARATION

8.2 WATER

SOP NO. 8.2.1, 8.2.2, 8.2.3, 8.2.4
DATE: 4/16/69
REVISION: 12/22/69

8.2.1 CONTAINERS

A square pack, one quart bottle with a 48 mm opening will be used for the water bottle.

8.2.2 FILLING

Seven bottles for each isolator and control cage will be filled to the one quart capacity with tap water.

8.2.3 STERILITY ASSURANCE

A batch number will be used for labeling which will correspond to numbers in a record book with the date of preparation and sterility test results.

The bottles will be placed in the autoclave so they are not touching one another and autoclaved for 30 minutes at STP.

The center bottle in the autoclave will contain a "Kilit"* ampule. In addition, 100 ml from the same bottle will be incubated with 200 ml of double strength Thioglycollate medium at 35°C for two weeks.

8.2.4 EMERGENCY RESERVES

At least 28 bottles of sterile tap water will be kept for an emergency reserve.

*Autoclave Sterility Control from Baltimore Biological Laboratory, Division Becton, Dickson Company, Box 175, Cockeysville, Maryland, 21030 (Stock #12018).

8. PRIMATE DIET PREPARATION

8.3 VITAMINS

SOP NO. 8.3.1, 8.3.2, 8.3.3, 8.3.4
DATE: 4/16/69
REVISION: 12/22/69

8.3.1 TYPE

Kor-Val Multi Vitamin Drops* will be used.

8.3.2 PREPARATION AND STERILIZATION

They will be diluted 1:1 with distilled water and sterilized through a 0.22 μ Millipore membrane filter.

8.3.3 CONTAINER FILLING AND DISPENSING

Appropriate bottles will be filled with the sterile vitamin solution in a laminar flow bench.

8.3.4 STERILITY ASSURANCE

Sterility checks will be done on each batch with at least five 1 ml aliquots each into 15 ml Thioglycollate tubes with incubation at 35°C for two weeks.

*E. J. Korvette Department Store, Route 202 & Gulph Road, King of Prussia, Pennsylvania 19406.

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.1 PREPARATION - GENERAL

SOP NO. 8.4.1
DATE: 4/16/69
REVISION: 12/22/69

1. All glassware and other items are washed in a suitable soap and hot water, rinsed in hot tap water three times and twice in deionized water and drained dry.
2. All glassware and material used in the isolator will be sterilized by an appropriate method, moist heat, dry heat, filtration, or gaseous ethylene oxide. The predominantly used agent will be moist heat. Autoclave times are as follows:

(a)	Water	60 minutes	15 lbs., 121°C (250°F)
(b)	Food	15 minutes	15 lbs., 121°C (250°F)
(c)	Glassware	20 minutes	15 lbs., 121°C (250°F)
(d)	Heparin sodium	20 minutes	15 lbs., 121°C (250°F)
(e)	Gloves and Toweling	20 minutes	15 lbs., 121°C (250°F)

3. Items that are sterilized by filtration (.22 μ filters)
   (a) 70% Isopropanol
   (b) Vitamins
4. Items sterilized by dry heat

(a)	Air Filter	2 Hours	175°C

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.2 PRE-PACKAGED ITEMS RECEIVED FROM VENDORS ARE ACCEPTED AS STERILE

SOP NO. 8.4.2
DATE: 4/16/69
REVISION: 12/22/69

1. Pre-packaged sterile syringes
2. Pre-packaged sterile gauze
3. Sernylan* (phencyclidine hydrochloride)
4. Prepared Culture Medium
5. Disposable pipetts
6. Four ounce fecal collection cups (Falcon Plastics #4013)
7. Sterile cotton applicator (Swubes - Falcon Plastics #2078)

*Parke-Davis Company, Detroit, Michigan

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.3 SALINE PREPARATION 9.0 ml AND 99.0 ml

SOP NO. 8.4.3
DATE: 4/16/69
REVISION: 12/22/69

Stock saline (0.85%) will be made and dispensed by means of an automatic pipettor (Brewer)* for 10 ml and 99 ml blanks. The containers will be 16 x 150 mm screw cap milk dilution bottles.

The saline will be sterilized by autoclaving 15 lbs., 20 minutes, 121°C (250°F).

The 99 ml glanks used with the fecal determination (SOP No. 3.2.4) are weighed before introduction of the fecal sample and weighed after the addition of the feces. This information is to be placed in the upper middle of Form #1 (SOP No. 3.3).

*Baltimore Biological Laboratory, Division of Becton, Dickson Company, Cockeysville, Maryland, 21030.

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.4 HEPARIN SODIUM PREPARATION

SOP NO. 8.4.4
DATE: 4/16/69
REVISION: 12/22/69

1. Heparin Sodium is prepared from crystaline form in a dilution of 500 units per 1 milliter of 0.85% saline.
2. Sterilized in an autoclave for 20 minutes, 15 lbs., 121°C (250°F) and dispensed in injectable bottles in a Class 100 Clean Bench.

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.5 HOCKEY STICK SPREADER AND STAINLESS STEEL SPATULA PREPARATION

SOP NO. 8.4.5
DATE: 4/16/69
REVISION: 12/22/69

The preparation of hockey stick spreaders and stainless steel spatulas are done in the same manner.

1. The items are washed in a suitable hot soapy water, rinsed with tap water three times and twice with demineralized water.
2. Packaged in Steriline Indicator* bags, and sealed with the triple fold gummed sealer.
3. The items are then autoclaved for 20 minutes, 15 lbs. pressure, 121°C (250°F) with a five minute drying time.

*Aseptic Thermo Indicator Company, through Joseph E. Frankle Company, Lab Supplies and Equipment, Philadelphia, Pa. (Cat. No. 276).

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.6 SCREW CAP BLOOD COLLECTING TUBES PREPARATION

SOP NO. 8.4.6
DATE: 4/16/69
REVISION: 12/22/69

1. Blood collecting tubes* (13 x 100 mm) are washed in hot soapy water, rinsed three times in tap water and twice in demineralized water.
2. The tubes are autoclaved while still moist with the screw cap loosened. The moisture generates steam on the inside of the tubes and the loosened caps allow for air and steam expansion.

*Fisher Scientific, Gulph Road, King of Prussia, Pa.

8. PRIMATE DIET PREPARATION

8.4 GLASSWARE AND MATERIAL

8.4.7 70% ISOPROPANOL PREPARATION

SOP NO. 8.4.7
DATE: 4/16/69
REVISION: 12/22/69

1. The 70% isopropanol is made from reagent grade isopropanol.
2. The alcohol is sterilized by filtration (.22 μ filter) and dispensed in sterilized bottles (SOP No. 8.4.1) in a Class 100 Blean Bench.
3. The screw cap bottles are stored in a designated cabinet set aside for sterile items.

8. PRIMATE DIET PREPARATION

SOP NO. 8.5
DATE: 4/16/69
REVISION: 12/22/69

8.5 RECORD KEEPING

Records will be kept in accordance with SOP Numbers 10.1, 10.4 and 10.5.

ISOLATOR - PREPARATION AND
MAINTENANCE

9. ISOLATORS - PREPARATION AND MAINTENANCE

SOP NO. 9.1
DATE: 4/16/69
REVISION: 12/22/69

9.1 GENERAL

The isolator is the most important equipment item in the testing program. It is the biological barrier or shield that maintains the conditions required for the success of the testing and reliability of the experimental results. It, therefore, must be assembled and maintained with the least amount of complication and without errors.

9. ISOLATORS - PREPARATION AND MAINTENANCE

9.2 INITIAL SET-UP OF ISOLATORS

9.2.1 PREPARATION OF FILTERS

SOP NO. 9.2.1
DATE: 4/16/69
REVISION: 12/22/69

The aluminum filter holder will be cleaned before addition of new filter material. Two individual, three layer thickness of 1/2 inch fiberglass filter material will be wrapped around the metal screen area. All seams will be taped with individual pieces of appropriate tape (tape that will survive the dry heat sterilization cycle).

The opening to the filter element will be sealed with a mylar cap and tape.

1. The filter is sterilized with dry heat oven at a temperature of 170°C for two hours.
2. An alternate sterilization method will be moist heat (30 minutes at STP) with a long vacuum-dry cycle (at least 30 minutes) to completely dry the wrapping and filter material.

A complete sterile filtration system will be maintained as a back-up for each isolator.

9. ISOLATORS - PREPARATION AND MAINTENANCE

9.2 INITIAL SET-UP OF ISOLATORS

9.2.2 INSTALLATION OF ISOLATOR COVERS

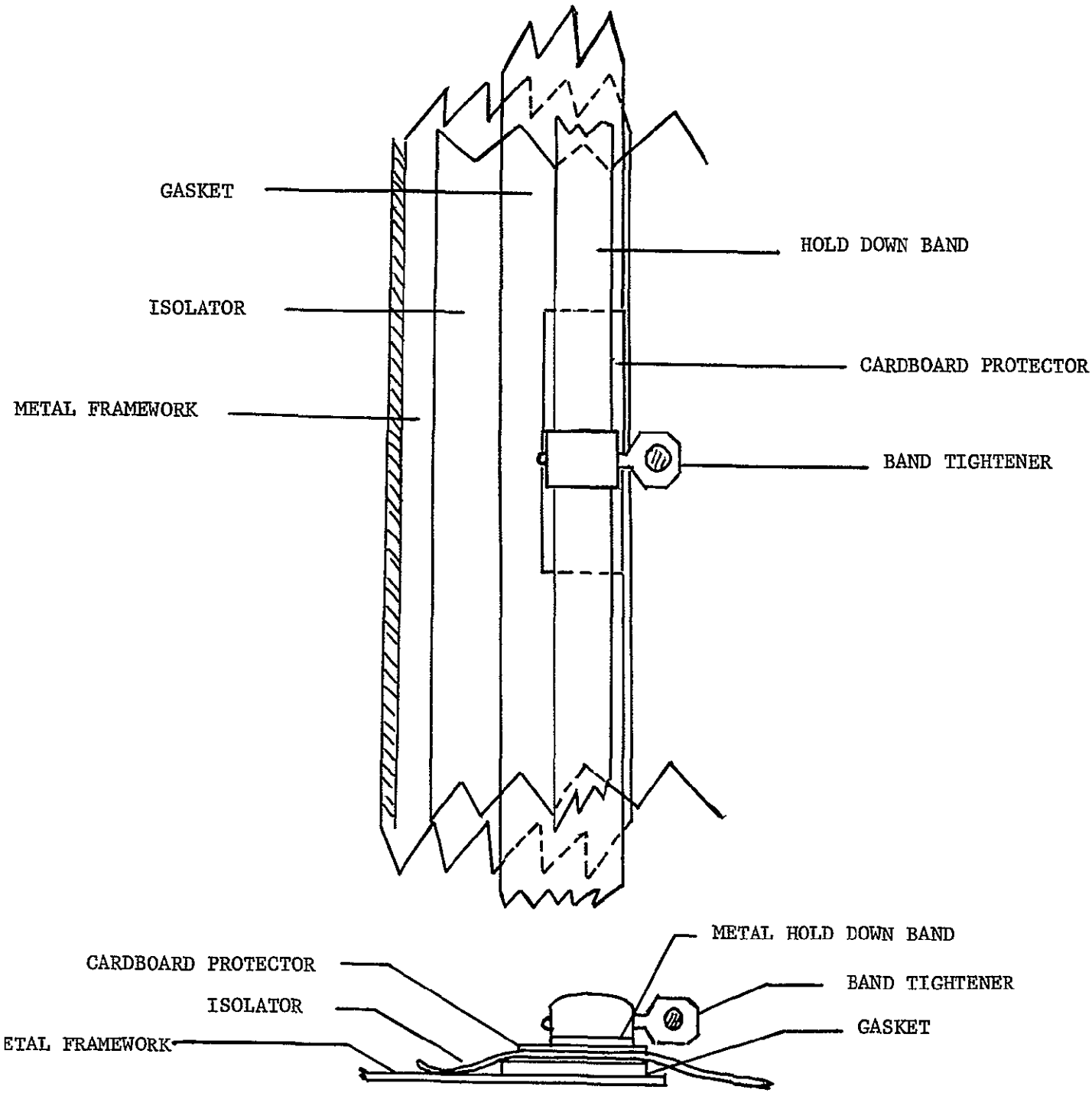
SOP NO. 9.2.2
DATE: 4/16/69
REVISION: 12/22/69

Install the 1" x 1/4" rubber gasket material* around the circular metal rims at each end of the isolator. Leave a 3 inch excess to allow for shrinkage. Carefully slide the isolator cover into position by placing sliding gromets on slide-rack into appropriate holes in isolator. Close the internal and external zippers by carefully sliding one and then the other a short distance at a time. Slide the cover onto the end gasket leaving a 1/2" overlap. Slip the metal hold-down band over the isolator and center it on the gasket. Before tightening, inspect the gasket and isolator making certain that unusual stress is not being applied at any one spot and that sufficient overlap remains over the entire circumference. Place a 1½" x 2" piece of light cardboard under the tightening-clamp to prevent the tearing of the plastic cover material when the clamp is tightened (see Figure 1). It is important to make sure that all folds are removed or flattened to prevent leaks and/or channels for leaks.

*Press-on Products, Inc, Addison, Illinois, 60101

FIGURE 1

DETAIL OF METAL HOLD DOWN BAND



9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.2.3
9.2 INITIAL SET-UP OF ISOLATORS	DATE: 4/23/69
9.2.3 LEAK TESTING AND REPAIR OF LEAKS AND TEARS	REVISION: 12/22/69

Extreme care must be taken to prevent leaks and tears from occurring. If any do occur, they should be repaired immediately with a bright colored plastic tape. This will mark the location for further refinement, if necessary, and is a reminder that the area should be checked for leaks at a later date. Inflate the isolator by operation of the circulator fan. Observe closely for any obvious leaks. Always maintain positive pressure on the system; this will obviate passage of contaminants through small holes.

Inspect the sleeve and glove areas:

(1) Isolator gloves and especially fingers may have small holes. These should be checked visually every time they are used.

(2) Rubber gloves should have outer and inner protective coverings, leather, etc. for outer and sterile surgical for inner.

(3) Carefully inspect the tape that fastens the gloves to the sleeves. Remove air bubbles and channels in the tape by pressing down and rubbing firmly with a piece of cotton.

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.2.4
9.2 INITIAL SET-UP OF ISOLATORS	DATE: 4/16/69
9.2.4 STERILIZATION OF ISOLATORS WITH PERACETIC ACID	REVISION: 12/22/69

Peracetic acid (2%) (SOP No. 9.3.5) is dispensed by plastic spray bottle covering all exposed surfaces with the liquid or gaseous vapor. The exposure time is 30 minutes with a desired relative humidity of between 40-60%. The system is then flushed with sterile air to evaporate the remaining liquid (peracetic acid and/or acetic acid and H_2O) and wash the vapors from the enclosed isolator. The flushing time continues until the escaping vapors do not contain the acid odors. The capacity of the isolator and air exchange rate determine the flushing time.

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.2.5
9.2 INITIAL SET-UP OF ISOLATORS	DATE: 4/16/69
9.2.5 QUALIFICATION OF ISOLATOR STERILITY	REVISION: 12/22/69

Random swab samples will be made (SOP No. 7.1) after sterilization and through flushing with air, placed in 15 ml liquid Thioglycollate medium incubated at 35°C for two weeks and observed for growth. If no growth occurs, the samples will be sub-cultured and the old samples inoculated with a low number of organisms to see if it will support growth.

9. ISOLATORS - PREPARATION AND MAINTENANCE

9.3 ROUTINE OPERATING PROCEDURES

9.3.1 DAILY CLEANING PROCEDURES

SOP NO. 9.3.1
DATE: 4/16/69
REVISION: 12/22/69

The isolators are kept in general "pick-up" order with all used items being removed and replaced by a new supply (SOP No. 7.2.5).

The control monkeys have their cages sprayed down with water to remove feces and urine collected in the catch tray. The catch tray has a continuous flow of water through it to wash excretion down the drain.

9. ISOLATORS - PREPARATION AND MAINTENANCE

9.3 ROUTINE OPERATING PROCEDURES

9.3.2 LEAK TESTING

SOP NO. 9.3.2
DATE: 4/16/69
REVISION: 12/22/69

A visual check for loss of pressure is performed periodically as the personnel are continuously working in the isolators. If a pressure loss is indicated, a quick search of suspect spots (zipper, gloves, etc.) locates the trouble spot.

9.	ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.3.3
9.3	ROUTINE OPERATING PROCEDURES	DATE: 4/16/69
9.3.3	REPAIR OF LEAKS	REVISION: 12/22/69

Leaks will be repaired according to SOP No. 9.2.3.

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.3.4
9.3 ROUTINE OPERATING PROCEDURES	DATE: 4/16/69
9.3.4 ISOLATOR BREAKDOWN OR OPENING PROCEDURES	REVISION: 12/22/69

The isolator is opened in the reverse order of SOP No. 9.2.2.

1. Monkey is transferred to the rigid small isolator.
2. Band tightener is loosened enough to allow the unzipping of the isolator bag.
3. The outside fabric zipper and the inside plastic zipper are opened in that order. Zippers will be operated carefully to prevent tearing of the zipper or isolator bag.
4. The plastic reinforcing straps will be tightened to support the lower half of the isolator.

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.3.5
9.3 ROUTINE OPERATING PROCEDURES	DATE: 4/16/69
9.3.5 PREPARATION AND USE OF PERACETIC ACID	REVISION: 12/22/69

1. Precautions: Peracetic acid (40%) should be stored in vented glass polyethylene containers in a cool place (refrigerator/freezer).
2. Handling and diluting equipment should be compatible with peracetic acid.
3. Surfaces should be washed before treatment so that they will be free of soil or dirt.
4. Peracetic acid should not be placed into potable water.
5. Dilute solution of peracetic acid, spray or vapors, are irritating to the eyes and nose. Contact of the liquid with the skin causes a dry yellow to brown layer to form on the area of exposure. This is not injurious as such but repeated contact will cause drying and eventual cracking of the skin.
6. If peracetic acid is to be used on new material, compatibility testing should be carried out with all necessary precautions.
7. Peracetic acid is a strong oxidizer and can react violently with certain substances.
8. Peracetic Acid (40% Solution)

   General Information:

   Synomyms: Peroxyacetic acid, acetyl hydroperoxide

   Description: colorless liquid; strong odor

   Formula: CH_3COOOH $O\text{-}CH_2\text{-}CH_2C = O$

   Constants: mol. wt: 76.05

   bp: $105^{\circ}C$ explodes at $110^{\circ}C$

   flash p: $105^{\circ}F$ (O.C.)

   d: 1.15 at $20^{\circ}C$

Hazard Analysis:

Toxic Hazard Rating

Acute Local:	Irritant - high, ingestion - high, inhalation - high
Acute Systemic:	Unknown
Chronic Local:	Unknown
Chronic Systemic:	Unknown

Fire Hazard: Dangerous

Explosion Hazard: Moderate, when exposed to heat or by spontaneous chemical reaction a powerful oxidizing agent.

Disaster Hazard: Dangerous, keep away from combustable materials.

A standard 2% solution is used for general decontamination of the isolators and materials to be transferred into the isolator.

The solution is prepared as follows:

30 ml Peracetic Acid (CH_3COOOH, 40% by weight)*
0.5 ml Naccanal 60 TL (55% triethanolamine alkyl aryl sulfonate)**
500 ml Distilled Water

Use of Peracetic Acid:

The use of Peracetic Acid is covered in SOP No. 9.2.4.

Individuals that use peracetic acid should adhere to the precautions listed in the first part of this section (SOP No. 9.3.5).

*Becco Chemical Division, Food Machinery & Chemical Corp., 633 Third Avenue, New York, New York 10017
**National Ancline Division, Allied Chemical Corp. 40 Rector Street, New York, N.Y. 10006

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.3.6
9.3 ROUTINE OPERATING PROCEDURES	DATE: 4/16/69
9.3.6 CHECK LIST OF ISOLATOR SUPPLIES FOR BACTERIAL AND SEROLOGICAL SAMPLES	REVISION: 12/22/69

1. Plastic shelf, cage bottom and urine collection system.
2. Four 1-liter bottles of sterilized water.
3. Two 1-gallon jars of sterilized food, (each jar contains 3 bags of food with 14 pellets per bag).
4. Four 1 cc syringes (sterile: pre-packaged).
5. Four 2½ cc syringes (sterile: pre-packaged).
6. Four sterile screw cap blood collection tubes (13 x 100 mm od) (SOP No. 8.4).
7. 100 ml of 1:1 diluted sterile vitamins (SOP No. 8.3) and medicine dropper bottle for application.
8. Sterile spatula (4" stainless).
9. Sterile Heparin Sodium 1:500 units (SOP No. 8.4).
10. Four 2" x 2" sterile gauze pads.
11. Spring scale in sterile container.
12. Two 8" x 12" plastic containers.
13. One sipper and rubber stopper for water bottle
14. Two pair of sterile cotton gloves.
15. 300 ml of 2% peracetic acid solution (SOP No. 9.3.5) in spray bottle for each isolator.
16. Four 2 ft. sq. sterilized cotton towels.
17. 100 ml of sterile 70% isopropanol.
18. One injectable vial of Sernylan* (phencyclidine hydrochloride).

*Parke-Davis Company, Detroit, Michigan.

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.4.1
9.4 MATERIAL TRANSFER - IN/OUT	DATE: 4/16/69
9.4.1 FOOD/WATER/VITAMINS/ISOLATOR SUPPLIES	REVISION: 12/22/69

All items to be transferred into the isolator are pre-sterilized and sealed (SOP No. 8.0). The sterilized containers are placed in the small rigid transfer box attached to the large isolator and sprayed with 2% peracetic acid solution. The isolator exposure lasts for 30 minutes (or longer if desirable). This is followed by a thorough flushing with air (SOP No. 9.2.4).

9. ISOLATORS - PREPARATION AND MAINTENANCE	SOP NO. 9.4.2
9.4 MATERIAL TRANSFER - IN/OUT	DATE: 4/16/69
9.4.2 BACTERIOLOGICAL/SEROLOGICAL ASSAYS	REVISION: 12/22/69

The insertion of items is covered in SOP No. 9.4.1

The removal of items is covered in SOP No. 7.2.5.

9. ISOLATORS - PREPARATION AND MAINTENANCE

9.4 MATERIAL TRANSFER - IN/OUT

9.4.3 CLEANING MATERIALS

SOP NO. 9.4.3
DATE: 4/16/69
REVISION: 12/22/69

The same or similar procedures are used that are carried out in SOP Numbers 9.4.1 and 9.4.2.

9. ISOLATORS - PREPARATION AND MAINTENANCE

SOP NO. 9.5
DATE: 4/16/69
REVISION: 12/22/69

9.5 RECORD KEEPING

The records will be kept in the same manner as SOP Numbers 10.1 and 10.2.

10. NOTEBOOKS/RECORD KEEPING

10.1 GENERAL CONSIDERATIONS

SOP NO. 10.1
DATE: 4/24/69
REVISION: 12/22/69

1. It is the responsibility of each individual to maintain complete, accurate, and up-to-date records of all data generated by operations under his/her control.
2. All entries will be made with a ball-point pen.
3. Erroneous entries, typographical mistakes, etc. will be crossed out with one line only through the inaccurate section, and the corrected portion written immediately following.
4. Conscientious effort will be made to write legibly, with each entry or observation written in clear, concise English. It is expected that all statements will be written so as to avoid ambiguity in order to prevent misinterpretations in the weeks or months following the entry.
5. All notebooks used will be the hard cover quadrille-ruled data books with preforated duplicate pages and which require a carbon insert. Exceptions to this will be the primary bacteriological isolation identification data and hematology data for which specially designed, specific data sheets will be provided.
6. It is understood that each person responsible for the separate record-keeping areas will be expected to furnish at regular intervals, summaries in rough form for inclusion within periodic reports to the customer.

10. NOTEBOOKS/RECORD KEEPING

10.2 ANIMAL AND ISOLATOR RECORDS

SOP NO. 10.2
DATE: 4/24/69
REVISION: 12/22/69

1. Data on each monkey together with his attendant isolator/cage will be kept in separate notebooks.
2. Entries will be made as a daily log beginning with the date, complete information, ending with the signature of the individual making the entry.
3. List of data to be included in these logs:
   (a) Pertinent, reasonably objective observations on the health or well-being of each monkey. Even if no obvious illness or problems develop, statements of the apparent good health of the animal should be entered at least every two weeks.
   (b) Each sampling and/or feeding period with observations on problems encountered, if any, and suggestions for improvements or possible solutions.
   (c) An estimation of the amount of water and food being consumed by the monkey between each feeding period.
   (d) All notations of air leaks, rips, tears, etc. to the isolators, and when and how they were repaired.
   (e) The results of all sterility control testing (as per SOP No. 9.3.3) performed on each isolator.
   (f) Date and description of each cleaning period.

10. NOTEBOOKS/RECORD KEEPING

10.3 BACTERIOLOGICAL DATA

SOP NO. 10.3
DATE: 4/24/69
REVISION: 12/22/69

1. Specific loose-leaf charts will be used to record all primary bacteriological sampling data in accordance with SOP No. 3.2.6.
2. Each chart will be inserted in a separate fiber-board binder, with a separate binder used for each monkey.
3. All summaries derived from the primary sampling data will be entered on specific summary charts made specifically for that purpose. These charts will also be kept in the separate notebook binders.
4. Each chart in each separate binder will be numbered consecutively with indelible markers as it is inserted in the binder.

10. NOTEBOOKS/RECORD KEEPING

10.4 SEROLOGY/HEMATOLOGY

SOP NO. 10.4
DATE: 4/24/69
REVISION: 12/22/69

1. Date of each bleeding, the method, site and the anticoagulant used will be entered on the loose-leaf chart provided.
2. Results with interpretations, together with adequate reference to procedures followed, will be included for each assay performed.
3. Any significant deviations or alterations from established procedures must be noted on the loose leaf sheet.

10. NOTEBOOKS/RECORD KEEPING

10.5 FOOD/WATER/VITAMIN STERILITY CONTROL

SOP NO. 10.5
DATE: 4/24/69
REVISION: 12/22/69

1. Records of food supplies, vitamins, and drinking water prepared for the monkeys and sterility tested according to SOP No. 8 will be maintained in a separate notebook.
2. The date of preparation and the date the results of the sterility tests were made will be recorded.
3. Any significant alterations in preparation or operation procedures will be made in the log.

11. SAFETY PROCEDURES

SOP NO. 11.1
DATE: 4/16/69
REVISION: 12/22/69

11.1 GENERAL

Safety is a very important aspect of any testing procedure. All persons will be thoroughly schooled in the functioning of all equipment, the handling of chemicals and the use of common sense safety.

Everyone will familiarize themselves with the location and operation of fire extinguishers. They will also learn the location of all electrical panel shut-offs.

The laboratory area (floor, sinks, tables, etc.) will be kept in an orderly manner and all refuse will be picked up and disposed of daily or as soon as containers become filled.

In the event of an accident (cuts, punctures, bites, etc.) the injured individual will immediately seek qualified medical attention. Company directives as to reporting are the responsibility of the Program Manager.

Laboratory Supervisor and Program Manager will be immediately notified of any accident involving laboratory personnel. Project Leaders (H. Kaplan and N. Behringer) are personally responsible for this item.

Physicals, to include x-rays of chest, are required of all personnel before beginning work and at intervals of not over six months.

11. SAFETY PROCEDURES

SOP NO. 11.2
DATE: 4/16/69
REVISION: 12/22/69

11.2 DISPOSAL OF VIABLE CULTURES AND CONTAMINATED MATERIALS

1. All glassware that has contained microorganisms will be autoclaved for 30 minutes, 15 pounds pressure at 121°C (250°F) prior to being discarded. Glassware consists of:
   (a) Petri Dishes
   (b) Pipetts
   (c) Test Tubes
   (d) Saline Blanks
   (e) Fecal Weighing Pans
   (f) Fecal Collecting Containers
2. Disposable items will be discarded and permanent glassware (test tubes, etc.) will be dumped and washed as in SOP No. 8.4.

11. SAFETY PROCEDURES

11.3 MALFUNCTION OF EQUIPMENT

SOP NO.	11.3
DATE:	4/16/69
REVISION:	12/22/69

SCOPE: All equipment on inventory including autoclaves, sterilizers, etc.

GENERAL: In the event of a known or suspected malfunction of inventoried equipment, apply the following procedure.

A. Determine seriousness or potential

1. Hazard
2. Damage
3. Effect upon immediate requirements (1 to 7 days)
4. Effect upon long term requirements (7 days to end of program)
5. Make a subjective judgement based upon 1, 2, 3, and 4 above and proceed as follows.

B. If damage is serious (presents immediate hazard to life and health of animals and personnel)

1. Locate (1) M. H. Bengson or alternate (2) J. A. Geating

   (1) Office: Extension - 2-5676 Home: 265-3249

   (2) Office: Extension - 2-6014 Home: TU 7-9235

2. Notify Patrol and describe hazard in personnel safety (This only if hazard could be seen to affect personnel who are not aware of nature of problem)
3. Take personnel precautions such as turning off equipment, posting notices, etc.
4. In no case endanger self!

C. If no hazard to personnel, but animal welfare is affected

1. Call M. H. Bengson or J. A. Geating
2. Take required steps to minimize or nullify hazard
3. In special case of power failure, broken pipes, etc. NOTIFY PATROL

D. In the event of non-emergency malfunction

1. Notify Dick Pease of Facilities - Extension 2-5183
2. Notify M. H. Bengson
3. Make appropriate notes in laboratory records
4. Consult SOP's such as isolator repair, etc.
5. Perform and document those repairs or actions taken such as placing OUT OF ORDER - DO NOT USE signs on equipment.
6. Within limits of vested authority, perform such repairs and actions as can be done safely. Document all actions.

   EXAMPLE: Failure of Deep Freeze

   ACTION: As above and transfer items to other deep freeze or close deep freeze and put on DO NOT OPEN sign, Date, Signature, Time and Reason.

E. Notify Laboratory Supervisor and make note in appropriate journal of all repairs and actions taken. Where possible, prepare paperwork such as work orders for signature of authenticating individual.

11. SAFETY PROCEDURES

11.4 TOXIC CHEMICALS - HANDLING AND PERSONNEL SAFETY

SOP NO. 11.4
DATE: 4/16/69
REVISION: 12/22/69

1. All general safety precuations will be used to prevent injury to individuals by hazardous chemicals.
2. Before handling of any chemicals, the manufacturer's specification will be consulted concerning their use and precaution to follow (see SOP No. 9.3.5).
3. If injuries occur from handling chemical or other items, the individual will seek immediate qualified medical help. If none is immediately available, they should perform immediate first aid (i.e., flush chemical burns with flowing water).
4. Proper protective clothing will be used when handling chemicals (lab coats, gloves, rubber aprons, etc.).

11. SAFETY PROCEDURES

11.5 MISCELLANEOUS

SOP NO. 11.5
DATE: 4/16/69
REVISION: 12/22/69

General Electric and Division Safety Policy Manual procedures shall apply. The Program Manager is appointed Safety Officer for the laboratory and shall schedule periodic Safety Meetings. All personnel will attend at least one such meeting every six months.

12. LABORATORY MAINTENANCE/SERVICES

12.2 DISHWASHING

SOP NO. 12.2
DATE: 4/15/69
REVISION· 12/23/69

1. Dishwashing will be done daily to prevent the accumulation of glassware and spent supplies.
2. Glassware and material will be cleaned as per SOP No. 8.4.
3. Materials and glassware will be stored in proper storage areas after drying.

12. LABORATORY MAINTENANCE/SERVICES

12.3 EQUIPMENT MAINTENANCE

SOP NO. 12.3
DATE: 4/15/69
REVISION: 12/23/69

The maintenance of equipment will be performed by only qualified persons. They may be company personnel or specially trained individuals who represent the vendor companies. The isolators and other specialized equipment used in the experimentation will be maintained or calibrated daily by operating technician with major problems being turned over to the manufacturer's representatives. Failure of equipment will be reported to laboratory supervisor. Electrical repairs will not be made without written authority of laboratory supervisor for each instance.

12. LABORATORY MAINTENANCE/SERVICES

SOP NO. 12.1
DATE: 4/15/69
REVISION: 12/23/69

12.1 GENERAL

The Laboratory will be kept in a general condition of "picked-up" at all times. If an area is used for experimentation or preparation, the area will be cleaned up prior to moving on to area of new operation.

12. LABORATORY MAINTENANCE/SERVICES

12.4 HOUSEKEEPING - DAILY/WEEKLY

SOP NO. 12.4
DATE: 4/15/69
REVISION: 12/23/69

The last hour of each working day will be devoted to the clean up of all areas of the laboratory. This will include the dumping of trash, washing of dishes, sweeping and/or mopping of the floors and general pick-up of the immediate working areas.

The floor will be mopped at least once weekly. All bench tops will be cleaned and thoroughly washed with an appropriate germicidal detergent once weekly.

Working areas will be washed with germicidal detergent prior to performing microbial assays or other experimental test where microbial contamination may effect the results.

GENERAL ELECTRIC
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